OP0064 GENETIC MARKERS FOR THE EFFICACY OF TNF BLOCKING THERAPY OF RHEUMATOID ARTHRITIS

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Background

Objectives The regulation of cytokine production on the level of the gene transcription may involve several types of polymorphisms in promoter/enhancer region. The aim of this study was to evaluate if polymorphism in several genes could associate with the responsiveness to TNF blocking therapy with etanercept (Enbrel®) in patients with rheumatoid arthritis from Eastern Sweden.

Methods 124 patients with active RA were treated with subcutaneous etanercept 25 mg twice a week. The efficacy measures included ACR and DAS28 response rates after treatment for 3 months. The ?308 TNFA, -1082 IL10 and codon 25 TGFB1 gene polymorphisms were detected by combination of PCR and restriction endonuclease mapping. VNTRs in intron 2 of IL1RN gene were detected by PCR and HLA-DQB1 and HLA-DRB1 were typed and subtyped by PCR-SSP.

Results 24 patients (19%) were non-responders according to both ACR and DAS28 criteria. We found significant association of one group, composite of TNF1/TNF1 and G/G genotypes, with positive responsiveness to etanercept ($p < 0.05$). Non-responders with TNF2 allele had relatively low ESR before treatment (mean $\pm$ SE = 25 ± 5 mm/min) in comparison with other groups ($p < 0.05$, Fisher's PLSD). Individuals with non-shared epitope genes showed approximately 2 times more frequent fail of the therapy if ACR criteria was used.

Conclusion We find strong evidence for association of certain genetic markers with efficacy of etanercept treatment in RA patients. Combined ?308 TNFA and ?1082 IL10 genotype may predispose for the response to etanercept therapy, while ?308 TNFA genotype in combination with ESR may be used for the selection of potential non-responders. These results may provide information for future improvement of treatment of RA.

OP0089 ASSOCIATION BETWEEN TNF -308A AND SYSTEMIC LUPUS ERYTHEMATOSUS IN RELATION TO HLA-DR3 AND SIX MICROSATELLITE MARKERS ON THE SHORT ARM OF CHROMOSOME VI

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Background Allelic imbalance at polymorphic loci within the human HLA-DRB1 and TNF genes has been observed in association with increased susceptibility to systemic lupus erythematosus.

Objectives We investigated whether the association of HLA-DRB1*0301 (HLA-DR3) and TNF-308A with SLE could be

attributed to linkage to six polymorphic microsatellites between HLA-DRB1 and HLA-C.

Methods Ninety-one consecutive Caucasian patients with SLE and 253 controls (organ donors) were typed for HLA-DRB1, D6S1014, D6S273, TNFA, MIB, C-1-2-5 and C-1-3-2 and for TNF promoter polymorphisms. Independent contribution of alleles to disease susceptibility was estimated by crosstabulation and multivariate regression.

Results Carriership of TNF-308A was associated with susceptibility to SLE (odds ratio [95% confidence interval], 3.70 [2.24–6.11]). This remained present after stratification on carriership of HLA-DR3 (pooled odds ratio, 2.53 [1.37–4.70]). Stratification further revealed a possible association of carriership of C-1-2-5*192 with protection from SLE beyond the effects of HLA-DR3 and TNF-308A. Gene dose effect was observed for -308A only (homozygotes, 7.75 [3.01–20.0], heterozygotes, 3.15 [1.85–5.37]). In multivariate analysis, the association between HLA-DR3, TNF-308A, and C-1-2-5*192 remained independently associated with susceptibility to SLE (2.58 [1.29–5.18], 2.76 [1.43–5.31], and 0.26 [0.10–0.66], respectively).

Conclusion An association of carriership of TNF-308A with susceptibility to SLE can not be attributed to linkage to HLA-DR3, nor to other polymorphic markers in the vicinity of the TNF gene. Further loci that are independently associated with SLE might be located in the vicinity of marker C-1-2-5.

OP0091 DIFFERENCES IN DISEASE EXPRESSION IN ADJUVANT ARTHRITIS OF LEWIS RATS INDUCED BY MYCOBACTERIA OR LIPOIDAL AMINE

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Background Marked polyarthritis is induced in male Lewis rats by injection of either heat-killed Mycobacterium tuberculosis in mineral oil (AdA) or lipoidal amine in complete Freund's adjuvant (LA/CFA). For both models, paw swelling and body weight loss occur in all rats by 10 to 12 days after immunisation.

Objectives Interestingly, however, the present study demonstrates that the morphologic and densitometric changes of AdA and LA/CFA are distinguishable.

Methods We induced AdA or LA/CFA in male Lewis rats (180 g) and evaluated both clinical (body and spleen weight, volume of the tibiotarsal [hock] joints) and morphologic changes (bone mineral density [BMD] by DEXA; histopathologic lesions in the hock and femorotibial [knee] joints). Readings were obtained at the day of onset (as determined by initial paw swelling) as well as 5, 10 and 25 days after onset.

Results Paw swelling, loss of body weight, enhanced spleen weight were identical in AdA and LA/CFA. In contrast, hock inflammation score, hock erosion score and hock cartilage score

Abstract OP0091 Table 1

	Control	AdA	LA/CFA
Hock BMD	100%	87.5%	99.9%*
Knee inflammation score	0	3.3 $\pm$ 0.5	2.2 $\pm$ 0.4***
Knee Erosion score	0	3.2 $\pm$ 1.2	1.7 $\pm$ 1.0***

*not different from control, ***different from AdA (Newman Keuls post hoc test).

were lower in LA/CFA, though not statistically significant from AdA. Lesions developed more slowly in LA/CFA than in AdA. However, hock BMD as well as knee inflammation and erosion scores found to be different (shown here at Onset + 5 days):

Conclusion Inflammatory and skeletal changes for AdA and LA/CFA were similar by clinical and histopathologic measures 10 days after onset, while inflammation receded by Onset + 25 days in LA/CFA more than it did in AdA. These data indicate that bone densitometric and morphologic findings can distinguish these two models of adjuvant arthritis despite their similar clinical presentations. This finding may be relevant in screening novel therapeutic candidates as these models may define different potential efficacious effects of a compound.

OP0093 INTERFERON GAMMA GENE POLYMORPHISM: RELATIONSHIP TO SUSCEPTIBILITY AND SEVERITY OF RHEUMATOID ARTHRITIS IN A PROSPECTIVE STUDY

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Background Interferon gamma (IFN γ) is a multifunctional cytokine that plays an important role in modulating most of the phases of the immune and inflammatory responses. IFN γ gene is a potential candidate as marker of rheumatoid arthritis (RA) susceptibility and severity.

Objectives In this prospective longitudinal study we have investigated the association between a variable length CA repeat in the first intron of the IFN γ gene and RA susceptibility and severity.

Methods One hundred and three early (< 1 year) RA patients (ACR 1987 criteria) were evaluated clinically, serologically and radiographically (modified Sharp/van der Heijde method) yearly for 4 years. The total radiological damage score was used to quantify RA severity after 4 years of follow-up. One hundred thirty unrelated healthy individuals were used as controls. The microsatellite region in the first intron of the IFN γ gene was analysed by PCR and electrophoresis in an automatic DNA sequencer.

Results Characteristics of the 103 RA patients at inclusion were as follows: 80 females, 23 males, mean age 49.4 ± 15.5 years, mean disease duration 6.9 ± 3.7 months, mean tender joint score 12.4 ± 8.9 , mean swollen joint number 6 ± 6.2 , mean erythrocyte sedimentation rate 29.8 ± 25.2 , rheumatoid factor positive (ELISA method) 71/103, mean radiological score 5 ± 13.6 . The allele sizes of the IFN γ gene polymorphic region ranged from 122 bp (11 CA repeats) to 134 bp (17 CA repeats). Twelve CA repeat (12R) and thirteen CA repeat (13R) alleles were the most common in RA patients (respectively 41.7% and 48.5%) and healthy controls (respectively 45.3% and 45.7%). 12R/12R, 12R/13R and 13R/13R genotypes were the most common in RA patients (respectively 15.6%, 44.1% and 21.6%) and healthy controls (respectively 22.4%, 38.0% and 21.7%). No association has been observed between the IFN γ gene polymorphism and RA susceptibility or severity.

Conclusion Although the present study fails to reveal any association between this particular IFN γ gene polymorphism and RA, the search for RA susceptibility and severity genes remains an important issue to better understand RA pathophysiology and to optimise the strategies for treatment of early RA.

OP0096 TRANSCRIPTOME ANALYSIS IN RHEUMATOID ARTHRITIS TREATED WITH A TNF NEUTRALISING TNF RECEPTOR: FUSION PROTEIN (ETANERCEPT)

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Background Treatment with etanercept (Enbrel) results in a rapid and sustained improvement of disease activity in patients with refractory rheumatoid arthritis (RA). Although changes in the expression of single proteins, particularly cytokines and proteases, could be demonstrated in the past at the protein and mRNA level, new methods are necessary to provide insight into the complex mechanisms which occur after neutralisation of TNF α .

Objectives Oligonucleotide arrays can provide a broad picture of the state of cells by monitoring the expression levels of thousands of genes at the same time. Here we report the application of DNA array technology (Affymetrix) to cover changes in the expression levels under etanercept treatment.

Methods Mononuclear cells (PBMC) and neutrophils (PMN) from peripheral blood were prepared applying standard procedures, synovial fluid (SF) was obtained from both knees. Total RNA for analysis was isolated from PBMC, PMN and SF-cells at different time points in the course of treatment.

Results The expression level of 5600 transcripts was analysed. The most expressed genes were found in SF-cells. About 3.3%, 2.4% and 0.2% of the assessed genes in PBMC, PMN and SF-cells, respectively, showed significant changes in gene expression in either direction (i.e. increase with a fold change ≥ 3 , decrease with a fold change $\leq 1/3$).

Conclusion The group of genes which are up- or downregulated included a number of genes which are known to be involved in transcription, chemotaxis, cell adhesion as well as genes not previously examined in the context of rheumatoid arthritis.

The results have to be considered preliminary and confirmed in further experiments.

OP0097 JUVENILE IDIOPATHIC ARTHRITIS (JIA) IS ASSOCIATED WITH THE D6S265 MICROSATELLITE POLYMORPHISMS ON THE HIGH-RISK DR8-DQ4 HAPLOTYPE

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Background Juvenile idiopathic arthritis (JIA) has been shown to be associated with at least four different alleles: HLA-A2, DR5 and DR6, and DP2. These associations are independent from each other, i.e. they are not caused by linkage disequilibrium between the markers. However, several studies have suggested that additional, unmapped, HLA-linked genes may participate in JIA.

Objectives The purpose of this study was to look for additional genes involved in the susceptibility for JIA in the HLA complex using microsatellite polymorphisms. Since it is known that JIA is strongly associated with the DR8-DQ4 haplotype only DR8-DQ4 haplotype was included in the study, in order to exclude associations secondary to this high-risk haplotype.