**Network:** Infection and Inflammation: from Pathogen-induced Signatures to Therapeutic Target Genes

**Project:** Clinical Validation by Custom cDNA Array

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**Introduction**

In Rheumatoid arthritis (RA), not only synovial tissue macrophages (Mφ) but also peripheral blood Monocytes (MO) are activated. Upon activation both cell types spontaneously release inflammatory cytokines and mediators, which in turn cause and sustain joint inflammation and destruction of connective tissue. Up to now, genes and pathways involved in RA monocyte activation are only partially characterized. In particular DNA microarrays are used to identify genes differentially regulated in RA. So far, these analyses identified: i.) RA disease relevant genes, ii.) M0/Mφ activation patterns in RA and in other chronic inflammatory diseases, iii.) Differentially regulated genes in anti-TNFα treatment with prognostic value, iv.) Pathways activated or blocked during different stages of the disease, and additionally v.) Potential therapeutic intervention sites. Based on these results a custom cDNA microarray was designed in NGFN-1. This array will be used within NGFN-2 for analysing RA patient MO blood samples for diagnosis, the detection of individual RA subtypes, and the responses of patients to different types of treatment. In parallel, the custom MO microarray will be expanded by genes altered in expression in synovial Mφ and T-cells isolated from RA patients.

**Results/Project Status**

Rheumatoid arthritis (RA) is a systemic inflammatory disease leading to joint destruction and ultimately loss of function. Especially cells of the monocyte/macrophage (MO/Mφ) system play a key role in mediating the course of RA. MO/ Mφ signature genes have been identified to be suitable for diagnosing the disease, its progression, and the success of treatment with TNFα (Adalimumab). Additionally, MO/ Mφ signature genes were found to be involved not only in chronic inflammatory diseases such as RA but also in other chronic inflammatory diseases, in cancer or in infectious diseases.

**Custom MO microarray and preselection of candidate genes for RA and therapy outcome**

These probes were identified in differentially hybridisation by gene subtraction of RA-MO versus normals. A further source of gene probes was whole genome analysis (U133A/B) of MO in samples from normals, RA patients prior to and during anti-TNFα treatment (n=7 each; Fig.1 and Fig.2).

In part, these patient samples and normals have been already analysed using Affymetrix U133A/B microarrays. All results obtained with the customised microarrays are in excellent agreement with previous analyses of normal and patient MO samples, and U937 cells. The gene selection of the candidate genes to diagnose RA disease and furthermore to calculate the the response of anti-TNFα treatment will be verified in close cooperation with the TP48 (Grützkau) and with the pharmaceutical industry partner Abbott. In this clinical study we will use a RNA probe set from patients prior to treatment and during therapy at 3 different time point (4weeks, 12w and 26w) from 30 patients and 15 healthy donors. Using this probe set too, genome

**Fig. 1: Application of PAM to identify genes determining response to anti-TNFα therapy.** A.) A threshold of 4.5 was applied. B) Probabilities for correct and mis-classification were calculated and identified treated patients TNF-4 and TNF-6 as RA-group. (ND = normal donor; TE = treated RA patient).

**Fig. 2: Hierarchical Clustering and molecular measures for response to anti-TNFα treatment.** A) Clustering of RA versus normal donor MO and B.) of RA versus ND and versus anti-TNFα treated patients.

Using this gene selection we designed a custom cDNA microarray. The unique collection of gene probes and the established SOPs for array fabrication, RNA isolation, cDNA synthesis and hybridisation provide the basis for the production of robust medically relevant data. Recently, a custom RA-MO microarray was established in close collaboration with the Kernbereich Plattform-2 (Max-Planck-Institute for Molecular Genetics; Lehrach, Hultschig; Berlin). This customised array contains RA relevant gene probes immobilised as denatured sequence verified PCR products (Fig.3). The successful design of the customised RA microarray for the detection of MO activation was verified by comparative analysis of non-stimulated and stimulated (LPS, PMA, Vit.D3+LPS, PMA+LPS) premonocytic U937 cells, and of non-stimulated and stimulated healthy donor MO. In addition, gene expression profiles of MO from RA patients prior to and during TNFα treatment were determined.

**Fig.3: RA-MO-Chipset-II — Complex hybridization A) 4x4 fields (–) were spotted, each containing 18*19 spots, including positive and negative controls. Each field contains the same set of genes and controls. B) Four types of subfields were produced with different arrangement of the spots to identify variability due to the spotting procedure.**

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Disease-oriented Genome Networks

Outlook

Microarrays are important, novel tools for characterization of disease activity and response to anti-TNFα therapy. They can be applied as screening systems for 1) diagnosis of arthritis, 2) therapeutic effectiveness, 3) investigation of drug effects, and 4) target identification for new therapeutics (anti-CD20; TP48, Grützkau). Thus, the experimentally defined current selection of genes on our cDNA microarray (RA-MO chip-set-II) could also contribute to the investigation of the role of MO in other rheumatic diseases and therapy studies, and to improve the understanding of regulated MO pathways. The RA-MO chip-set-II is cost-effective, competitive, flexible, and applicable for different MO/Mψ-oriented questions in RA, infectious disease, or other states of chronic inflammation. The customized microarray will be expanded by other candidate genes from synovial tissue (Häupl, Berek) and also from MO (TP48, Grützkau). Furthermore, several other functionally unknown candidate genes will be characterized by siRNA knockdown strategies. The will be to investigate their pathway regulation and function in chronic diseases or cancer. Several patents national and international patents were submitted. Cooperations with the pharmaceutical industry might open new avenues to define novel drugs for effective treatment and hopefully will force the national economics.