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

Project: Therapeutics-induced Gene Signature in Chronic Rheumatoid Diseases

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Introduction

In rheumatoid arthritis many pro- and anti-inflammatory cytokines were identified as being overexpressed in the bloodstream and the rheumatoid synovium. Especially, TNF-alpha were ascribed pro-inflammatory effects which were accompanied by clinical complaints, such as pain, fatigue and mobility. It could be shown by numerous clinical studies including more than 500.000 patients (the majority treated for rheumatoid arthritis) that the pharmacological strategy to neutralize TNF-alpha will improve these clinical complaints. The anti-inflammatory effect evoked by anti-TNF therapeutics is explained by the central function of TNF to orchestrate the recruitment of leukocytes into joints, upregulation of adhesion molecules, chemokines and proinflammatory cytokines, such as IL-1 and IL-6. The clinical effectiveness comprises approximately 60%-80% responders independent of which neutralizing anti-TNF antibodies (Infliximab, Adalimumab) or soluble TNF-receptors (Etanercept) are applied. Until now, the reasons responsible for the non-responsiveness are poorly understood and at the moment no reliable molecular or clinical marker are available which may help to predict the therapeutic success. The complexity of the mechanism of action of anti-TNF-therapies is also reflected by the side-effects which are different after treatment with anti-TNF antibodies and soluble TNF-receptors.

Another promising therapy is based on the deprivation of B lymphocytes by application of a CD20-specific antibody (Rituximab). Besides its benefit in patients with non-Hodgkin's lymphoma, in RA patients, Rituximab also improved clinical complaints, such as synovitis. In contrast to the short-lasting effects of TNF-blocking therapeutics a single application of Rituximab causes a sustained reduction of B lymphocytes for approximately six months. Since B cells are not only responsible for humoral immune responses, but may also function as antigen presenting cells, it would be interesting to get informations whether monocytes were also functionally influenced by this kind of therapy. This assumption is also supported by the observation that Rituximab treatment is paralleled by increased TNF-alpha levels in the serum of patients suffering large B-cell lymphoma.

Project Status

Cell sorting

The concept to analyse purified cell populations by the microarray technology revealed convincing advantages in comparison to whole blood samples in the context of data interpretation. In NGFN-1, standardized cell sorting procedures were established which enabled us to isolate up to 10 highly purified leukocyte populations from 50-100 ml peripheral blood for gene expression profiling experiments. This complex procedure as illustrated in Fig. 1 has been optimized according minimization of artificial induction of gene expression, purity and viability of cells sorted.

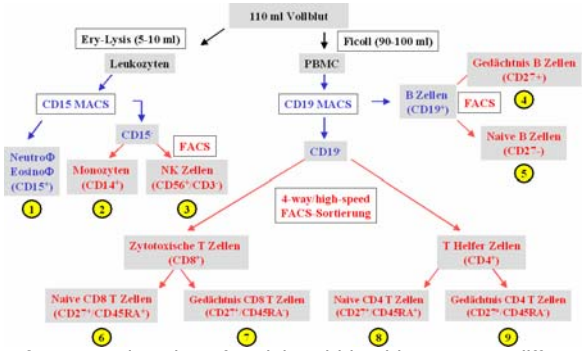

Fig 1: *Fractionation of peripheral blood in up to ten different leukocyte populations by the combination of magnetic (MACS) and fluorescence activated cell sorting (FACS).*

According to this procedure purified cell populations from healthy donors, rheumatoid arhtritis (AS), ankylosing spondylitis (AS) and systemic lupus (SLE) patients before therapy and after treatment were collected for gene expression analyses.

Gene expression profiling

Gene expression profiling will be done by the Affymetrix technolgy which has been established in NGFN-1. By now we have determined cell type-specific gene expression profiles and identified disease-specific signatures for monocytes of RA-, AS-, and SLE patients (Fig. 2).

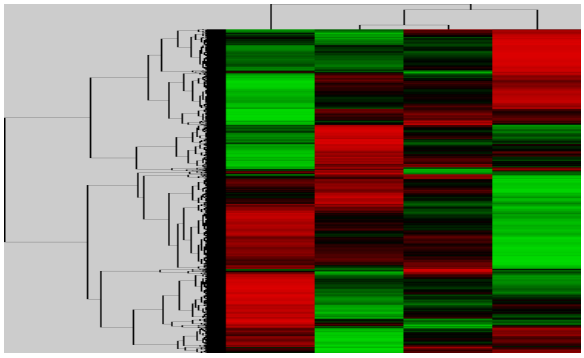

Fig 2: *Classification of ND- (n=10), RA- (n=8), AS- (n=12) and SLE-monocytes (n=9).*

At the moment the disease-classifying potential of monocyte signatures will be compared to signatures obtained from other immune cells, such as CD4- and CD8-lymphocytes.
The signatures will also be compared to signatures obtained from monocytes confronted with defined cytokine- and drug-signals ex vivo, and macrophages from inflamed tissue, to identify the underlying pathways and novel molecular targets (TP49 Baumgrass).

For the identification of therapy-induced signatures and prognostic markers for the evaluation of therapeutic success, the gene expression studies will focus primarily on peripheral monocytes and T helper cells of RA- and AS-patients.

In Fig. 3A and 3B the successful classification of patients before and after anti-TNF-treatment is shown. Fig. 3A represents similarities between patients before and after treatment according to clinical parameters. The patients RAantiTNF4 and RAantiTNF6 showed no improvement under therapy and therefore were grouped in proximity to the untreated patients.

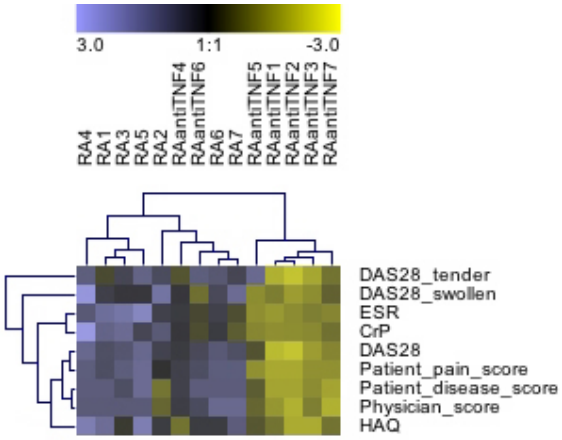

Fig 3A: *Clinical classification of RA samples before and after anti-TNF-α treatment. Each row represents a clinical parameter used as a disease score. Each column indicates an individual included in the study.*

An almost similar classification pattern was obtained if the expression profiles of monocytes were compared (Fig. 3B). The untreated patients formed one major cluster together with the non-responders RAantiTNF4 and RAantiTNF6 and the anti-TNF-treated patients formed the other cluster in proximity to the normal donors.

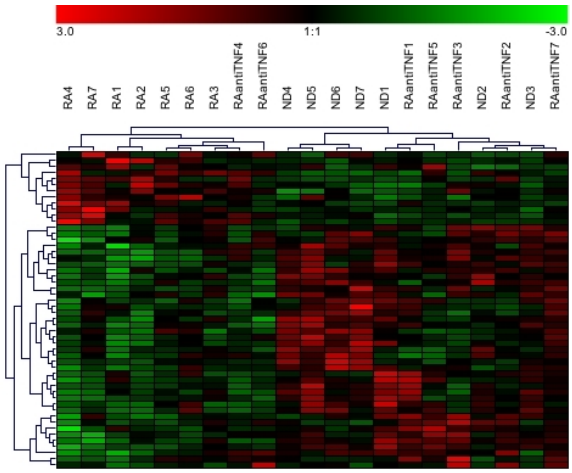

Fig 3B: *Hierarchical clustering of differentially expressed genes of ND and RA samples before and after anti-TNFα treatment. Each row represent a gene and the columns show the expression of 52 differentially regulated genes expressed by each individual in the study. Red indicates genes that are expressed at higher levels compared with the control mean. Green indicates genes that are expressed at lower levels relative to the control mean.*

Some of the candidate genes obtained by gene expression profiling have been successfully validated by real-time PCR in close cooperation with the TP29 (Stuhlmüller).

Outlook

The identification of prognostically valuable marker genes may be used in advance to predict individually whether an anti-TNF or anti-CD20 therapy will be successful or not. This will be done by the customized arthritis chip (TP29 Stuhlmüller). Candidate genes characterized so far will be spotted as cDNA or oligomer probes to generate a novel custom microarray. In parallel cytometric profiling of proteins encoded by the candidate transcripts will be tested as marker molecules to obtain a fast and cheap screening approach which might be useful for diagnosis and therapy monitoring.

Finally, the signatures obtained will be compared to signatures derived from cells confronted with defined cytokines and drugs in vitro (TP49 Baumgrass) to identify the pathways involved, relevant signals, and thus new candidate target genes with therapeutic potential.