

**Network: Infection and Inflammation: from Pathogen-induced Signatures to Therapeutic Target Genes****Project: Comparative Expression Profiling of T-cell Subsets and Monocytes and Analysis of Cell Type Specific Gene Function in Mouse Models of Chronic Inflammation****Alexander Scheffold - German Arthritis Research Centre (DRFZ), Berlin - scheffold@drfz.de****Alf Hamann - Humboldt University Berlin - Charité****Martin Lipp - Max-Delbrück-Center, MDC, Berlin****Introduction**

Chronic inflammatory diseases like Rheumatoid Arthritis or chronic infections are associated with the accumulation of large numbers of lymphoid and myeloid cells at the site of inflammation. Inflammatory T cells and monocytes are critically involved in the pathogenesis of the disease whereas regulatory T cell populations counteract immunopathology and bear considerable therapeutic potential. The various populations in peripheral blood as well as in inflamed tissue can be identified on the basis of functional or phenotypic markers. The identification of genes that contribute to the differentiation or effector function of these protective and inflammatory subsets is a decisive step towards new therapeutic approaches for chronic inflammatory diseases.

During NGFN-2 we aim at the functional characterization and validation of genes associated with the pathophysiological signature of chronic inflammation. This subproject will contribute to a comparative analysis of clinical data and data from well characterized experimental models of chronic inflammation, based on the knowledge about critical T cell subsets and monocytes. We will compare cell-type specific gene expression profiles obtained from *in vivo* models for chronic inflammation or from cells stimulated *in vitro* with specific inflammatory triggers with the expression profiles of cells from healthy controls generated during NGFN-1. This will allow a more reliable identification of genes associated with distinct stages of inflammation and infection and the responsible triggers. The results will be compared to data obtained from the human system and with data from infectious diseases generated within the network to identify common and disease-specific pathways, genes and relevant therapeutic targets, both in mice and men. Candidate genes will be functionally analyzed in *in vivo* models of chronic arthritis based on adoptive transfer of transfected cells, which allows genetic knockdown or overexpression of candidate genes in specific cell types. The use of techniques for the functional analysis of target molecules, such as the siRNA-approach, will allow us to dissect pathophysiological processes down to the molecular level and to define new targets for therapy.

**Aims:**

- (i) To validate cell-specific expression profiles generated during NGFN-1 and to generate additional profiles from the relevant cell types isolated *ex vivo* from an animal model of arthritis and trigger-specific signatures (in collaboration with SP "cytokine signatures")
- (ii) A comparative bioinformatic analysis of gene expression between
  - *ex vivo* disease versus control mice versus trigger-specific signatures
  - mouse versus man

- (iii) To identify common and disease specific signatures in chronic inflammation and infection and to correlate these signatures to specific triggers (by comparison with other groups from the network).
- (iv) To identify universal gene expression profiles in mouse and man (by comparison with other groups from the network).
- (v) A functional analysis of the populations identified here *in vivo* and *in vitro*
- (vi) A rapid functional screening of candidate genes in the adoptive transfer model of antigen-induced arthritis, by genetic inactivation or ectopic overexpression.

**Project Status****Generating disease, trigger and cell-specific expression profiles**

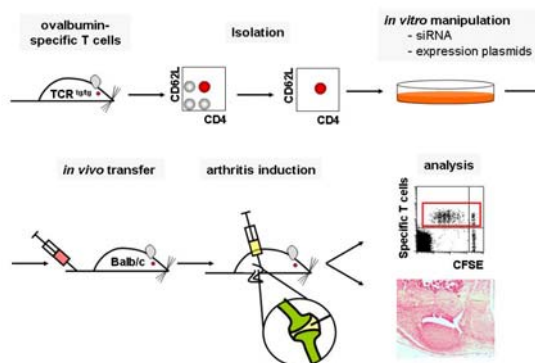
Based on our results in NGFN-1 we analyze the composition of the CD4+ T cell compartment at different stages of antigen-induced arthritis corresponding to the acute, early chronic and late chronic phase of the disease. We have already defined specific marker combinations for the identification of functionally distinct subsets of T cells, e.g. the chemokine receptors CCR7 and CXCR5, secreted cytokines such as IL-10 or IFN- $\gamma$ , CD25, and the integrin  $\alpha_E\beta_7$  or the E- and P-Selectin ligands which mediate homing into inflamed tissues. Monocytes and selected subsets of T cells have been isolated by FACS and MACS and used for microarray analysis to generate cell-type specific gene expression profiles. The expression profiles will be compared to the profiles already obtained during NGFN-1, e.g. from healthy control mice. In addition, we will isolate the respective cell populations from healthy animals and stimulate them with specific inflammatory triggers, e.g. TNF- $\alpha$ , IL-1, IL-12, IL-15, IL-17, and chemokines, to generate trigger-specific gene signatures (in collaboration with SP "trigger specific signatures").

These trigger, stage and subset specific gene signatures shall be compared with gene expression data for human cells and/or mouse models of infectious diseases. Together with other members of the network we have already generated a large number of control signatures during NGFN-1. In this way, we will identify genes that are critical in chronic inflammation and that are potential candidates for disease diagnosis and therapy. The bioinformatic analysis and the integration of data from other subprojects will be carried out by our central bioinformatics unit and in collaboration with the SMP Bioinformatics.

### Functional analysis of candidate genes and cell populations *in vitro* and *in vivo*

Subsets of T cells expressing genes with a critical role in disease development or progression shall be evaluated *in vitro* and *in vivo*. RNA expression levels of candidate genes will be validated by real-time PCR and protein expression patterns evaluated by FACS analysis or immunohistochemistry. To precisely define the role of these genes and cell subsets we can use several model systems of chronic inflammation (antigen-induced arthritis, colitis, DTH) and infection (viral, bacterial, parasites) that are already established in our laboratories or are available within the network. For the rapid functional screening of candidate genes within these populations we have established e.g. a cell-type specific *in vivo* system (fig.1). The critical cell population will be transfected retrovirally or by using the recently developed Nucleofector technology (Amaxa GmbH) *in vitro* and subsequently transferred *in vivo* into arthritic mice at distinct stages of the disease. By adoptive transfer of modified antigen-specific T cell populations we can analyse the functional consequence of the modification on the antigen-specific immune response within a normal animal.

Using the Amaxa Nucleofection system we could show that siRNA can directly be transfected into murine CD4 T cells with an efficiency of 100%. We achieved a specific knockdown of up to 90% on the mRNA and 70-80% on the protein level. The effect was stable for up to 5 days in *in vitro* cultured cells. By knocking down GATA3, the Th2 directing transcription factor, in these primary murine CD4 T cells we could demonstrate a reduced frequency of IL-4 producing cells upon *in vitro* culture, demonstrating that this new technology can be used to directly analyse gene function in primary T cells *in vitro*. Currently we are establishing the conditions for testing the modified T cells in *in vivo* disease models.



**Fig 1:** Experimental system for the functional gene analysis in T cells in *in vivo* disease models. T cells derived from TCR-tg/tg mice can be transfected *in vitro* to overexpress or knockdown a previously identified target gene. The modified T cells populations can be transferred into non-transgenic cells and the effect of the gene modification on the antigen-specific immune reaction and disease development can directly be analysed.

Currently similar systems are established for the analysis of genes affecting antigen-presenting cells such as monocytes or dendritic cells. In first experiments we were able to specifically knockdown IL-12 by *in vitro* transfection of dendritic cells via gene-gun vaccination using an siRNA expression plasmid against IL-12 p35. The IL-12 knockdown resulted in strongly reduced levels of antigen-specific IFN- $\gamma$  production, proving the functional knockdown of the gene *in vivo*.

### Outlook

With the now established functional *in vivo* screening system we will analyse several target genes which have been identified during NGFN-1 and the last year. We analyse the influence of these genes on arthritis induction and maintenance. The analysis of genetically modified Treg cells, which have strong protective capacity in this *in vivo* arthritis system will be used to define the critical protective genes, required for immune suppression *in vivo*.

The technical system can also be transferred to other disease models and thus can be used by several groups within the NGFN-network. It provides a rapid and efficient way for screening of gene function in primary cells *in vivo*.