

**Network: Infection and Inflammation: from Pathogen-induced Signatures to Therapeutic Target Genes****Project: Inflammatory Pathways in Human Disease: Definition of Specific and Common Mechanism of Tissue Response**Matthias Kretzler - Ludwigs Maximilians University, München – [matthias.kretzler@med.uni-muenchen.de](mailto:matthias.kretzler@med.uni-muenchen.de)**Introduction**

The human organism has developed a complex response pattern to tissue damage with disease specific and common response elements. The goal of this network is the definition of human tissue response to inflammatory stimuli. Gene expression profiles will be obtained from renal biopsies of autoimmune disease, ischemia-reperfusion damage, alloreactivity in renal transplant rejection and pathogen-induced damage. The expression profiles will be compared to lupus, rheumatoid and septic arthritis tissue to identify disease and tissue specific mechanism will be identified.

The human organism has developed a complex response pattern to tissue damage and inflammation, with elements specific to the noxious stimuli and common response pattern. The human kidney is exposed to the full spectrum of environmental challenges and could therefore serve as a paradigmatic organ system to elucidate the spectrum of inflammatory responses common or specific to the different inflammatory challenges. Using a worldwide unique renal biopsy RNA bank (the European Renal cDNA Bank, ERCB) established in Munich, large cohorts of renal tissue allow a genome wide expression analysis of the inflammatory response in

- Autoimmune disease of lupus nephritis in systemic lupus erythematoses.
- Alloreactivity in acute and chronic, cellular and humoral renal transplant rejection.
- Hypoxic tissue stress of ischemia-reperfusion damage in renal transplantation
- Pathogen induced tissue damage in viral and bacterial kidney infection.

The identified molecular pathways will be related to analysis of primary non-inflammatory renal diseases of hypertensive nephrosclerosis and diabetic nephropathy. In parallel, expression profiles of identical disease in different organ systems generated at the Berlinflame unit for lupus, rheumatoid and septic arthritis will allow a further refinement of disease specific progression pathways across tissue boundaries. The expression profiling will be matched with a genome wide functional screen for novel inflammatory markers. Using a unique genome wide overexpression technology, soluble factors activating common downstream inflammatory response in intrinsic renal cells will be identified and related to the expression regulation in inflammation. The further definition of identified novel pathways will use established *in vitro* systems of intrinsic and infiltrating renal cells and transgene models combined with murine renal damage models.

**Project Status**

The identification and definition of the role of novel mRNAs requires a comprehensive data pool based upon an organ specific tissue bank with sufficient sample size. In a DHGP and EU Framework V supported large international multicenter study such a renal biopsy repository could be established for frozen tissue. In the human renal biopsy core facility more than 1700 biopsies representing all relevant renal disease categories have been assembled and this frozen tissue can effectively be used for a genome wide expression scan. The tissue bank currently receives material from Europe, the US (including NIH studies) and southeast Asia for centralized processing and analysis. In addition, screening for candidate genes can be performed in a paraffin

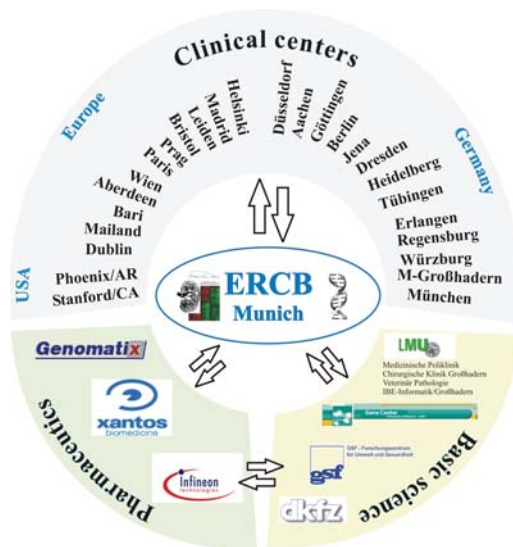
biopsy archive of more than 10.000 patients at the DKFZ in Heidelberg.

**Standardization:**

Concerning the sample acquisition standard operating procedures have been implemented in all 24 participating centers, establishing a robust and reproducible protocol for tissue and clinical data acquisition. Ethical approval and informed consent is available according to the local regulations at each university. Standardized histological analysis is performed by a core group of reference nephropathologies coordinated by the DKFZ. Follow-up data of disease course are obtained in 12 monthly intervals, allowing the correlation of the molecular phenotype with progression and therapeutic response pattern. Microdissection and compartment specific analysis of the ERCB and archival biopsies enables the characterization of nephron segment specific gene expression regulation, i.e. glomerular microvasculature and tubulo-interstitial compartments. Proprietary linear amplification technologies allow oligonucleotide based gene expression profiles to be obtained in a reproducible pattern. The currently Affymetrix based analysis passed all relevant quality controls with a minimal inter-assay variation after two-fold linear amplification and a stringent correlation of the array data compared to real-time RT-PCR based independent mRNA quantification (Ernst et al. 2002).

**Molecular disease classification**

In an EU FP V framework a comprehensive expression analysis has been obtained from diabetic nephropathy, minimal change disease, thin basement membrane disease, ischemia-reperfusion damage and several control populations. As the expression data sets allow a molecular classification of the tissue according to disease entity and severity the feasibility of the approach has been demonstrated. In addition common and disease specific molecular pathways could be extracted from this data set (Henger et al. 2004). Defined pathways activated in renal disease could be extracted using novel pathway mapping tools (i.e. Wnt /TGF-beta pathway in diabetic nephropathy). The identification of transcriptional regulation modules as potential key drivers of the disease process helped to reduce the complexity of the data set further. A supplementary strategy for gene expression analysis on human tissue allows the study of gene expression on formaldehyde fixed and paraffin embedded tissues after laser capture microdissection. The method enables the selection of vascular compartments and nephron segments with defined histological structure and enables mRNA analysis even after years of storage. In the department of Molecular and Cellular Pathology at the German Cancer Center (DKFZ) a comprehensive tissue archive with more than 1500 biopsies per year has been collected over the last ten years. This archival tissue bank can also be used for mRNA and protein tissue localization of candidate genes. The department has a proven record for sensitive immunohistochemistry and immune-electron microscopy. All clinical data, morphometric histological analysis and gene expression pattern are stored in a central database. As a consequence of the gene expression core facility character of the Munich unit, there is a tract record of over three years in effectively generating and distributing disease specific gene expression data for a large consortium.



**Fig 1:** Overview of the interactive network of the European Renal cDNA Bank.

### Outlook

With the relevant technologies in place, comprehensive gene expression analysis has been initiated. Using the human kidney as a paradigmatic organ system for the spectrum of inflammatory responses the tubulointerstitial and glomerular compartments of autoreactive inflammation, alloreactive inflammation, hypoxic tissue stress and pathogen induced tissue damage will be analyzed.

The expression data from the different inflammatory conditions will allow to dissect pathways specific for the different stimuli and pathways constituting a common response pattern. Expression profiles will be collated using gene ontology approaches and pathway mapping software. The analysis of different nephron segments in the array experiments has been shown to significantly reduce complexity in our hands. The transcriptional regulatory modules responsible for the pathways identified will be characterized using proprietary software tools. The pathway analysis will primarily focus on the area of expertise in the network, mainly established and novel innate and adaptive immune response pathways. In addition, this data set will serve as a resource for network members to screen molecules found to be regulated in *in vitro* settings and animal models for differential regulation in human renal disease. First data indicate the feasibility of this approach, as inflammation has been identified as a driving force in progressive diabetic nephropathy with significant induction of mRNAs specific for the innate and adaptive immune response. Differential mRNA expression will be reconfirmed using established human real-time RT-PCR in LCM from formalin fixed archival tissues (DKFZ Heidelberg) of glomeruli, renal microvasculature and interstitial compartments, followed by immunohistochemistry. The unit at the DKFZ has a considerable track record for staining of chemokines and innate immunity molecules.

### Pathway analysis

The pathway analysis will concentrate on the innate immune response and the adaptive immunity. For further functional analysis *in vitro* culture system of all relevant intrinsic renal cells are available. The renal cells can be combined with infiltrating macrophages and T-cells in co-culture system or complex tissue engineering techniques establishing an *in vitro* filtration barrier to analyze functional cross-talk of these cells. For *in vivo* characterization, transgene animal models can be challenged using an array of murine renal damage models (murine *lpr/lpr* lupus nephritis, folic acid induced

acute tubular necrosis, unilateral ureter obstruction and renal transplant rejection) to establish causal relationships.

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