Infectious versus immune mediated interstitial nephritis - deep analysis of leukocyturia and renal tissue to improve differential diagnosis and therapy

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Background and preliminary data:

Interstitial nephritis (IN) is a common cause of renal failure with many different underlying etiologies. These include drug hypersensitivity reactions as well as autoimmune diseases such as Sarcoidosis, Sjögren´s Syndrome and inflammatory bowel disease (IBD). Infections are another frequent cause of IN. The nephropathology department of the UKE sees more than 30 cases annually, where an infectious etiology is suspected, as especially ascending bacterial infections and Hantavirus. The diagnosis of IN in general, as well as the differential diagnosis of immune- versus infection- related IN remains a challenge, since clinical presentation is non-specific and there are no established biomarkers. In order to be able to study IN pathophysiology in more detail, we have recently set up a specialized outpatient clinic with currently more than 120 IN patients. The diagnostic gold standard for IN is renal biopsy. However, even histopathology is often unable to differentiate between the different causes. In particular, IN associated with IBD closely resembles infectious IN. Unfortunately, in many cases, even urinary culture and PCR-analysis of the specific bacterial ribosomal subunits from renal tissue do not allow unambiguous classification. Since the competing immunological and infectious etiologies require different therapeutic measures, quick differentiation is of high clinical importance. As a measure to improve time-to-diagnosis, we have established a flow cytometry based protocol to analyze leukocyturia. Preliminary data show, that urinary $CD4^+$ T helper cells (Th) predominate in immune mediated forms of IN, while in infectious IN, we find more neutrophils and $CD8^+$ T cells. In analogy to the different urinary leukocyte profile, we suspect differing molecular mechanisms underlying destruction of renal tissue in the different pathogen associated forms of IN versus immune mediated IN. In order to address this aspect, we have started to routinely perform flow cytometric, single cell RNA (scRNA-seq) as well as single nuclear RNA (snRNA-seq) analyses of renal biopsies from IN patients. In addition, we have been able to establish a protocol to generate organoids of renal tubuli from the urine of individual patients with IN of all subtypes. Bulk RNA-seq analyses of these tubuloids were performed to identify disease specific alterations of tubulus biology. In this respect, preliminary data point towards increased activation of leukocyte/tubulus adhesion, rather than increased renal leukocyte recruitment, at least in some forms of IN. We aim to evaluate, if this holds true for all IN etiologies, or whether some causes (e.g. specific infections or particular autoimmune diseases) show other predominantly activated pathways. Furthermore, we have been able to

perform leukocyte/tubuloid co-cultures, which showed that diseased tubulus cells indeed activate $CD4^+$ Th cells and in the case of IBD-IN support Th17 polarization. It will be interesting to find out, if different forms of infectious and immune mediated IN will result in expansion of different Th cell subtypes. In summary, the current project proposal aims to improve diagnosis of IN, facilitate differential diagnosis of immune versus infection related forms and identify shared as well as IN subtype specific pathways, which could possibly serve as therapeutic targets.

Hypothesis:

- The mechanisms of renal injury in IN differ between the different types of infectious and immune mediated diseases
- Urinary leukocytes can differentiate infectious from immune mediated IN
- IN subtype specific alterations of renal tubulus cells play an important role in renal inflammation
- Modulating the interaction between leukocytes and tubulus cells represents a novel therapeutic strategy

Aims and Work Programme:

- **Aim #1:** To evaluate urinary leukocytes as novel biomarkers for diagnosis and surveillance of infection related versus immune mediated IN
- **Aim #2:** To identify and characterize shared and distinct molecular profiles in infection related and immune mediated forms of IN
- **Aim #3:** To identify functionally important pathways in different IN subtypes via analysis of patient derived tubuloids and co-culture with leukocytes

Aim #1: Analyses of Leukocyturia by flow cytometry

1. We will aim for a deeper analysis of urinary leukocytes in general and particularly Th cell phenotypes by establishing FACS staining for markers of Th1 (CXCR3, Tbet), Th2 (Gata3), Th17 (CCR6, RORγt) cells, Tregs (Foxp3, CD25, CD127), naïve/memory T cell subsets (CD45R0, CD45RA, CD44, CD69, CD62L), Th cell proliferation (Ki-67) and co-stimulatory pathways (PD-1, CTLA-4). We expect to find different patterns of urinary Th cells in immune versus infection related forms of IN.
2. We will extend our study cohort and aim to recruit approximately 15 patients from each disease group as well as 20 healthy controls. Longitudinal analyses of urines from these patients before and during therapy will show, whether leukocytes and particularly Th cells can be used as marker for the presence of IN, differentiate between infectious and immune mediated causes and predict response to therapy.

Aim #2: Deep molecular profiling of renal tissue from IN patients

1. We will extend our FACS and scRNA-seq data, to advance our understanding of renal infiltrating leukocytes in immune versus infection related IN with a focus on Th cells and their key effector cytokines as e.g. IFNγ, IL-17A&F, TNFα, IL-22, IL-4, IL-13. Since our preliminary data point towards high T cell receptor clonality in immune-mediated IN, we will perform further T cell receptor analyses to find out, whether or not this is also the case in bacterial or virus associated IN. The phenotypes of the clonally expanded cell populations will be characterized in more detail to find out, whether they differ according to the underling IN subtype.
2. We aim to refine our analyses from snRNA-seq data and characterize leukocyte/resident renal cell interactions with a focus on chemokines (in particular CXCL5, CXCL9, CXCL10, CXCL13, CCL2, CCL5 and their receptors) as well as leukocyte adhesion pathways (as e.g. VCAM-1, ICAM-1). Also, since first data indicate particularly strong activation of the local complement system in infection related IN, we will analyze activation of renal cell intrinsic C3 and C4 complement pathways. Finally, our snRNA-seq analyses have enabled us to identify specialized interstitial renal endothelial cells, which line the peritubular capillaries and are thus likely to participate in renal leukocyte extravasation. Since virtually nothing is known about this celltype in inflammation so far, we will study specific alterations in immune versus infection related IN.
3. We will perform spatial transcriptomics of IN biopsies to study compartmentalization of renal leukocytes and integrate the data with our RNA-seq analyses. In particular, we aim to study different distribution of leukocytes to different anatomical niches of the renal cortex area versus infiltration of the renal medulla. The latter seems to be a particular hallmark of hanta virus associated IN for currently unknown reasons.

Aim #3: Generation and analysis of renal tubuloids from IN patients

1. We aim to in depth analyze bulk RNA-seq data to identify disease specific alterations. In particular, we will study tubulus cell activation and differentiation pathways, chemokine production and expression of adhesion molecules (e.g. VCAM-1, ICAM-1). Furthermore, we will analyze expression of potentially tubulus protective molecules, such as the EGFR ligand family (in particular amphiregulin/AREG, HBEGF, EGF and Betacellulin) as well as the cytokine IL-15 and its receptor.
2. We will analyze tubuloid/leukocyte interactions by in vitro co-cultures to define individual contributions of tubulus cells versus leukocytes to IN. The effects of tubuloids from healthy donors on leukocytes will be compared to those mediated by tubuloids from patients with different forms of IN. In particular, we will analyze Th cell phenotypes by flow

cytometry (activation and proliferation markers as well as expression of lineage markers as e.g. Tbet, RORyt and cytokines including IFNγ, IL-17, IL-4).

3. We will perform neutralization studies to test the significance of potential key molecules for tubulus/leukocyte interactions in IN. Currently, promising candidates are VCAM-1 and ICAM-1.

4. We will analyze tubuloid morphology from the different forms of IN by immunohistochemistry. Since our preliminary data have shown pathologic alterations of the tubular basement membrane in tubuloids derived from IN patients, we will elaborate on this aspect, which might play a key role in leukocyte/tubulus cell interactions.