**Project: Infections in autoimmune liver disease**

Project leader: Dr. med. Britta F. Zecher, I. Department of Medicine, University Medical Centre Hamburg-Eppendorf and Leibniz-Institute of Virology, Department of Virus immunology[1,2]
PD Dr. med. Johannes Hartl, I. Department of Medicine, University Medical Centre Hamburg-Eppendorf[1]

Affiliation: [1]I. Department of Medicine, UKE; [2]Leibniz Institute of Virology

**Background and preliminary data:**
Autoimmune hepatitis (AIH) is a rare autoimmune disease, affecting approximately 20 in 100,000 individuals with a strong female predominance. Individuals with AIH require long-term immunosuppressive treatment to control disease activity, prevent organ damage and progression to liver cirrhosis. Main side effects of immunosuppressive treatment include increased risk of malignancy and infection. Under first-line treatment with azathioprine, opportunistic infections such as Candida infection or reactivation of cytomegalo virus (CMV) or varicella zoster virus (VZV) can occur [1]. Vaccination studies demonstrated that immune response after vaccination is reduced in individuals with AIH independent of immunosuppressive treatment [2,3] – which is explained to be linked to a phenomenon described as endogenous immunosuppression that can be observed also in other forms of autoimmune disease. However, to date there is very limited evidence on how immunosuppression translates to clinical relevant risk of infections in autoimmune liver disease. Defining susceptibility towards infectious disease as part of clinical risk assessment is highly relevant in the field of solid organ transplantation, where immunosuppression is required to prevent organ rejection. In this context, *torque teno* viremia has been studied as a surrogate marker for immunosuppression. *Torque teno virus* (TTV) belongs to the family of *anelloviridae* that are part of the human virome and are acquired early in life. In the immunocompromised host, there is a significant increase in *anellovirus* DNA load, which has been described to be associated with levels of drug-induced immunosuppression in individuals after organ transplantation, with azathioprine co-treatment leading to elevated levels of TT viremia. Data on TT viremia in individuals with autoimmune hepatitis are scarce. Elevated levels of TT viremia can however be found in advanced liver disease and after liver transplantation [4,5]. Furthermore, TT viremia was described to be negatively correlated with lymphocyte count, suggesting that standard immunosuppressive treatment in AIH could associate with increased *anellovirus* DNAemia.
Preliminary data indicate increased rate of lymphopenia and anellovirus DNAemia in individuals with AIH compared to controls.

**Hypothesis:**
***We hypothesize that anellovirus DNAemia can serve as surrogate for the immune status and risk of infection in individuals with autoimmune hepatitis with and without immunosuppressive treatment.*** We will address this research question in the following aims.

**Aims and Work Programme:**
1. To assess frequency of common infectious diseases and respective risk factors in individuals with autoimmune liver disease and controls
2. To study anellovirus DNAemia as correlate for immune status in individuals with autoimmune liver disease and controls

**In Aim #1,** we will perform a questionnaire-based survey to collect information on frequency of common infectious diseases and risk factors (ID-survey; supplementary information 1) among individuals with AIH, individuals with PBC – chronic autoimmune liver disease not requiring immunosuppressive treatment – and healthy controls will serve as controls. All three

groups will be matched with regard to age and sex. The groups of individuals with AIH and PBC will be furthermore matched for stage of chronic liver disease. or individuals with AILD, we will collect clinical information on stage of liver disease, disease activity and immunosuppressive treatment as part of the routine clinical visit (supplementary information 2). Clinical data will be recorded in a separate questionnaire by the treating physician. All data of individuals with AILD will be processed in a pseudonymized form. The patient questionnaire reflects frequency and severity of infections within 12 months prior to the survey.

**In Aim #2,** blood samples will be additionally collected, in a subset of individuals with AILD participating in the ID-survey, PBMCs and plasma are isolated from EDTA-treated blood. To determine the immune status of participants, we will analyze PBMC samples for frequencies, absolute numbers, differentiation and activation status as well as functional properties of lymphocyte populations. This will be complemented by analysis of plasmatic cytokine profile. Furthermore, quantitative pan-*anellovirus* PCR will be performed from plasma of individuals with AILD to determine *anellovirus* DNAemia.
We will correlate susceptibility toward infections and disease severity determined via the ID-survey as well as frequency and phenotype of lymphocyte populations to *anellovirus* DNAemia. We thereby aim to identify surrogate parameters for infection susceptibility in individuals with autoimmune liver disease to help guide clinical risk assessment and patient counselling.
After two to three years of stable remission of under immunosuppressive treatment, well-controlled immunosuppression withdrawal can be offered to individuals with autoimmune hepatitis. The clinical rationale is to prevent life-long immunosuppression and associated risk of infections and malignancy, if stable remission can be maintained in the absence of immunosuppressive treatment. Reduction or complete withdrawal of immunosuppressive treatment, however, is associated with the risk of disease relapse. Under reduction of immunosuppression, individuals with AIH are therefore closely monitored at our outpatient clinic. Respective clinical samples have been collected in the Livernet biobank. We will longitudinally analyse clinical surrogate markers for drug-associated immunosuppression such as lymphocyte count and drug metabolites during immunosuppression withdrawal. *Anellovirus* DNAemia will be quantified and correlated the respective parameters of drug-associated immunosuppression. Furthermore, individuals with AIH will be divided into two groups based on their clinical course in individuals with stable remission (persisting normalisation of transaminases and IgG) or relapse. This will allow us to analyse *anellovirus* DNAemia with respect to disease status and individual markers of disease activity (transaminases, IgG). We thereby aim to determine if increase of disease activity associates with increase of *anellovirus* DNA as potential surrogate parameter for endogenous immunosuppression in active inflammation.

**Project-related publications:** (max. 5)


[1] Kirchgesner J, Lemaitre M, Carrat F, Zureik M, Carbonnel F, Dray-Spira R. Risk of Serious and Opportunistic Infections Associated With Treatment of Inflammatory Bowel Diseases. Gastroenterology 2018;155:337-346.e10. https://doi.org/10.1053/j.gastro.2018.04.012.

[2] Lohse AW, Kögel M, Meyer zum Büschenfelde KH. Evidence for spontaneous immunosuppression in autoimmune hepatitis. Hepatology 1995;22:381–8.

[3] Duengelhoef P, Hartl J, Rüther D, Steinmann S, Brehm TT, Weltzsch JP, et al. SARS-CoV-2 vaccination response in patients with autoimmune hepatitis and autoimmune cholestatic liver disease. United Eur Gastroenterol J 2022;10:319–29. https://doi.org/10.1002/ueg2.12218.

[4] Rueschenbaum S, Ciesek S, Queck A, Widera M, Schwarzkopf K, Brüne B, et al. Dysregulated Adaptive Immunity Is an Early Event in Liver Cirrhosis Preceding Acute-on-Chronic Liver Failure. Front Immunol 2020;11:534731. https://doi.org/10.3389/fimmu.2020.534731.

[5] Engel B, Görzer I, Campos-Murguia A, Hartleben B, Puchhammer-Stöckl E, Jaeckel E, et al. Association of torque teno virus viremia with liver fibrosis in the first year after liver transplantation. Front Immunol 2023;14:1215868. https://doi.org/10.3389/fimmu.2023.1215868.