by significantly increased IL-4 and GM-CSF cytokine production compared to arthralgia subject ($P < 0.001$ and $P = 0.01$) and RA-patient ($P < 0.001$ and $P = 0.004$) synovial tissue. However, not all polyfunctional T-cells are equal in their pathogenic potential. Therefore, in order to identify highly pathogenic synovial T-cells, cluster analysis of flow cytometric data using the unsupervised algorithm Flow-Som was performed and led to the identification of specific T-cell clusters with unique polyfunctionality characteristics. Specifically a cluster of CD4⁺CD8⁺ double positive (DP) T-cells with high polyfunctionality scores was identified. Hybrid flow cytometry and imaging technique confirmed the co-expression of CD4 and CD8 by a synovial T-cell population. DP T-cells are enriched in RA-patient synovial fluid and synovial tissue and arthralgia subject synovial tissue, but are absent from HC synovial tissue. Importantly, DP T-cell synovial accumulation strongly ($P = 0.002$) correlates with DAS28(CRP) of RA-patients. Initial studies utilising the novel, non-invasive FLIM technique for visualisation of cellular NAD, revealed that DP T-cells have a metabolic profile indicative of activated memory T-cells.

Conclusion: These data highlight a key early loss of balance between protective and pathogenic synovial T-cell polyfunctionality and the emergence of specific, highly polyfunctional and pathogenic T-cell clusters in RA.

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POS0008

IDENTIFICATION AND CHARACTERIZATION OF HISRS+ CD4⁺ T CELLS PATIENTS WITH IDIOPATHIC INFLAMMATORY MYOPATHIES

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Background: Idiopathic inflammatory myopathies (IIM) also known as myositis, are rare chronic autoimmune disorders which are represented by muscle weakness and extra-muscular features such as skin rash, interstitial lung disease (ILD) and arthritis. One of the most common autoantibodies in myositis, with a prevalence of 25-35%, is the anti Jo-1 autoantibodies, targeting the histidyl-transfer RNA synthetase (HisRS). Although the exact mechanism of how these antibodies are developed is unknown, we have previously shown that upon stimulation of both peripheral blood mononuclear cells (PBMC) and bronchoalveolar lavage fluid cells (BALF) with HisRS protein, CD4⁺ T cells were activated and produced inflammatory cytokines. Hitherto the presence of antigen specific autoreactive T cells has not been established in myositis, however previous studies by our group showed a strong indication of their presence with a reactivity to a specific HisRS peptide.

Objectives: The main aim of this project is to detect and characterize HisRS specific CD4⁺ T cells using HLA Class II tetramers. HLA Class II tetramers allow the detection of rare antigen specific CD4⁺ T cells and are widely used in studies of immunity, vaccine development, allergy monitoring and in autoimmunity. These cells are of specific interest to understand autoimmunity and to develop new therapies in autoimmune diseases

Methods: HLA-DRB1*03:01 monomers with selected tetanus and HisRS peptides were in-house in *E.coli* system. The peptides of interest were attached to the N-terminus of the HLA b-chain via a flexible peptide linker. HLA-tetramers were assembled using a commercial fluorescently labeled streptavidin. The efficacy of the peptide-HLA tetramers was validated by stimulating PBMCs from HLA-matched healthy controls with tetanus peptide. The frequency of tetanus specific CD4⁺ T cells were detected at different time points (6,13 and 21 days) from the cultures using tetanus peptides bound HLA-DRB1*0301 tetramers. The presence of tetanus specific T cells was confirmed by the secretion of significantly higher IFN γ levels upon re-stimulation of cells with tetanus peptide. The same protocol is applied for the HisRS-peptide tetramers. Peripheral blood cells are analysed from anti-Jo1+ and HLA-DRB1*0301 positive patients with IIM.

Results: Applying this method, our preliminary findings demonstrate the presence of HisRS+CD4⁺ T cells in peripheral blood from Jo-1+ patients (n=3) using HisRS tetramers following stimulation with the respective peptide. We are now including more patient samples to confirm our findings, and further characterize their phenotype and functionalities by flow cytometry and ELISA/fluorospot assays

Conclusion: Myositis is a rare and chronic autoimmune disorder, with no currently available cure. Previous studies indicate the importance of T cells in this disease. However, the phenotype, functionality and role of these cells in the

disease pathogenesis has not been fully established. Characterization of this autoreactive T-cell population will help us enhance our understanding of the disease pathogenesis and thus to develop better treatment options.

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POS0009

THE RELATIONSHIP BETWEEN DIFFERENT IGG AND IGA ANTI-MODIFIED PROTEIN AUTOANTIBODIES IN RHEUMATOID ARTHRITIS

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Background: Seropositive rheumatoid arthritis (RA) is characterized by the presence of rheumatoid factor (RF) and anti-citrullinated protein autoantibodies (ACPA) with different fine-specificities. Yet, other serum anti-modified protein autoantibodies (AMPA), e.g. anti-carbamylated (Carb), anti-acetylated (KAc), and anti-malondialdehyde acetaldehyde (MAA) modified protein antibodies, have been described. By using RA patient single-cell derived monoclonal antibodies we have previously shown that individual ACPA clones recognize small distinct citrulline-containing epitopes giving them extensive multireactivity when these epitopes are found in many peptides and proteins. Moreover, certain CCP2+ multireactive ACPA clones bind also to carbamylated and acetylated autoantigens [1].

Objectives: To provide a comprehensive evaluation of serum IgG and IgA auto-reactivity to different post-translational modifications in RA.

Methods: We analyzed 30 different IgG and IgA AMPA reactivities to modified antigens by ELISA and autoantigen arrays, in N=1985 newly diagnosed RA patients and population controls. The study utilized both previously established (i.e. IgG and IgA CCP2; IgG ACPA fine-specificities; IgG anti-Carb fibrinogen and Carb FCS; IgG and IgA Cit/Carb/KAc/Orn(Ac)-vimentin), and novel assays (e.g. IgG anti-MAA and IgG anti-acetylated histones). Association with patient characteristics such as smoking and disease activity were explored. The newly developed assays were also evaluated in SLE disease controls and CCP2+ RA-risk individuals without arthritis.

Results: Carb and KAc reactivities by different assays were primarily seen in patients also positive for citrulline-reactivity. Modified vimentin (mod-Vim) peptides were used for direct comparison of different AMPA reactivities, revealing that IgA AMPA recognizing mod-Vim was mainly detected in subsets of patients with high IgG anti-Cit-Vim levels and a history of smoking. IgG acetylation reactivity was mainly detected in a subset of patients with Cit and Carb reactivity. Anti-acetylated histone 2B reactivity was RA-specific and associated with high anti-CCP2 IgG levels, multiple ACPA fine-specificities, and smoking. This reactivity was also found to be present in CCP2+ RA-risk individuals without arthritis. Our data further demonstrate that IgG autoreactivity to MAA was increased in RA compared to controls with highest levels in CCP2+ RA, but was not RA-specific, and showed low correlation with other AMPA. Anti-MAA was instead associated with disease activity and was not significantly increased in CCP2+ individuals at risk of RA. Notably, RA patients could be subdivided into four different subsets based on their AMPA IgG and IgA reactivity profiles.

Conclusion: We conclude that autoantibodies exhibiting different patterns of ACPA fine-specificities as well as Carb and KAc reactivity are present in RA and may be derived from multireactive B-cell clones. Anti-Carb and anti-KAc could be considered reactivities within the "Cit-umbrella" similar to ACPA fine-specificities, while MAA is distinctly different.

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