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Background: Aberrant activation of B cells and autoantibody mediated tissue damage are hallmarks of autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Therefore, novel treatments that prevent autoantibody generation or antibody-mediated end organ tissue damage are of high interest. Bruton's tyrosine kinase (BTK) transduces signals downstream of the B cell receptor (BCR), toll-like receptors, and Fc receptors in B cells and myeloid cells [1]. Overexpression of BTK in B cells leads to hyperactive BCR signaling, plasma cell generation, autoantibody secretion, and an SLE-like disease in mice [2]. Conversely, reducing BTK expression in B cells can ameliorate disease in Lyn-deficient mice.[3] BTK inhibitors, such as evobrutinib, have entered clinical studies for the treatment of autoimmune diseases.[4]

Objectives: Small molecule-induced protein degradation offers a unique approach to target BTK; this approach simultaneously eliminates both BTK kinase activity and BTK-mediated scaffolding interactions in the signalosome. Chimeric Targeting Molecules (CTMs) are small molecules that catalyze ubiquitylation and proteasomal degradation of target proteins and are comprised of a ubiquitin ligase binding element ("harness"), a linker, and a target binding element ("hook"). NX-5948 is a CTM that contains a BTK hook linked to a cereblon (CRBN) harness. We examined the activity of NX-5948 in a collagen-induced arthritis model as part of an assessment of its potential as a drug candidate for autoimmune disease.

Methods: Cellular degradation of BTK, Aiolos and Ikaros as well as induction of CD69 and CD86 was determined using flow cytometry. Degradation of BTK in CD-1 mice or cynomolgus monkey was determined using flow cytometry analysis. In a collagen-induced arthritis (CIA) model, mice were vaccinated with type II collagen and treated before the onset of symptoms. Serum cytokine and anti-type II collagen antibody levels were determined using Luminex and ELISA, respectively.

Results: In human PBMCs, NX-5948 degrades BTK at sub-nanomolar concentrations and inhibits BCR signaling as measured by CD69 and CD86 induction in anti-IgM-stimulated B cells with similar potency. Oral administration of NX-5948 in mice leads to BTK degradation to <10% of baseline levels in circulating and splenic B cells. NX-5948 also promotes potent BTK degradation in cynomolgus monkeys, and it can suppress BTK levels to <10% of baseline levels after a single oral dose as low as 10 mg/kg.

Unlike IMiD drugs such as lenalidomide, the CRBN harness of NX-5948 was designed to avoid the degradation of known CRBN neo-substrates Aiolos (IKZF3) and Ikaros (IKZF1). In primary human T cells, NX-5948 induces minimal degradation of Aiolos and Ikaros and does not promote IL-2 secretion suggesting that NX-5948 does not convey IMiD activity associated with agents such as lenalidomide.

We examined the activity of NX-5948 in a mouse CIA model compared to that of the BTK inhibitor ibrutinib or dexamethasone as a positive control. In mice treated with NX-5948, symptoms of arthritis were resolved, and a significant reduction in arthritis clinical score was observed. Treatment with NX-5948 resulted in a reduction in anti-type II collagen titer and serum levels of the pro-inflammatory cytokine IL-6. Treatment with NX-5948 yielded superior anti-inflammatory activity relative to ibrutinib and similar activity to dexamethasone. Treatment with NX-5948 was well-tolerated and, unlike dexamethasone, did not promote body weight loss.

Conclusion: Degradation of BTK by NX-5948 shows robust activity in a CIA model compared to existing agents tested as controls. These findings provide support for further investigation of NX-5948 in additional models of autoimmune disease to inform plans for clinical development.

REFERENCES:

- [1] Crofford et al. 2016. Expert Rev Clin Immunol 12: 763–773.
- [2] Kil et al. 2012. Blood 119: 3744–3756.
- [3] Whyburn et al. 2003. J Immunol 171: 1850–1858.
- [4] Haselmayer, et. Al. 2019. J Immunol 202: 2888–2906.

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POS0007

LOSS OF BALANCE BETWEEN PROTECTIVE AND PRO-INFLAMMATORY SYNOVIAL TISSUE T CELL POLYFUNCTIONALITY PREDATES CLINICAL ONSET OF RHEUMATOID ARTHRITIS

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Background: Effective treatment of Rheumatoid arthritis (RA) patients is achievable within a short window of opportunity following diagnosis. T-cells are early drivers of synovial inflammation of RA, therefore, identification of pathogenic T-cell subsets at the synovial tissue of pre-RA, arthralgia subjects, would greatly improve our understanding of disease pathogenesis. Comparative analysis of healthy control, arthralgia subject and RA-patient derived synovial tissue T-cell responses will lead to the identification of pathogenic as well as protective cytokine milieu, thus enabling the identification of early therapeutic targets to help steer the immune response towards resolution.

Objectives: Characterization of T-cell polyfunctionality in the periphery and synovial tissue of 'at-risk' subjects (Arthralgia) RA-patients and healthy controls (HC).

Identification of specific, pathogenic, synovial tissue T-cell subsets.

Methods: Synovial biopsies from RA, AR and HC were obtained by arthroscopic surgery followed by RNAseq analysis (Guo et al., PLoS One, 2018). Single cell synovial tissue cell suspensions from RA, AR and HC and paired PBMC were stimulated *in vitro* and polyfunctional synovial T-cell subsets examined by flow cytometric analysis, SPICE visualization and FlowSom clustering. Flow-Imaging, was utilised to confirm specific T-cell cluster identification. Fluorescent Lifetime Imaging Microscopy (FLIM) was used to visualise metabolic status of specific T-cell populations.

Results: T-cell associated pro-inflammatory gene pathways were increased in RNAseq analysis of RA-patient and arthralgia subject compared to HC synovial tissue biopsies. Flow cytometric analysis of pro-inflammatory cytokine (TNF- α , IFN- γ , IL-2, GM-CSF, IL-17A, IL-22) production and SPICE analysis of *ex vivo* stimulated T-cells revealed marked polyfunctionality of arthralgia subject synovial T-cells, thus providing evidence for a dysregulated synovial T-cell response that pre-dates clinical onset of disease. Importantly, HC synovial tissue harbours a small, albeit surprisingly polyfunctional, CD4 T-cell population characterised

Figure 1. Identification of highly polyfunctional and pro-inflammatory synovial DP T-cells. A. Cluster analysis of RA-patient synovial tissue T-cells (asterisks indicate DP T-cell clusters). B. Flow imaging of CD4⁺, CD8⁺ and DP synovial T-cells. C. SPICE flow cytometric data visualization of DP arthralgia subject and RA-patient synovial T-cells. D. Correlation between the frequency of RA-patient synovial DP T-cells and disease severity.

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.

REFERENCES:

- [1] Sahlström P, Hansson M, Steen J, Amara K, Titcombe PJ, Forsström B, Stålesen R, Israelsson L, Piccoli L, Lundberg K, Klareskog L, Mueller DL, Catrina AI, Skriver K, Malmström V, Grönwall C. Different Hierarchies