SAT0031

VITAMIN B3 (NAM) SUPPRESSES T CELL ACTIVATION IN AND PRODUCTION OF PRO-INFLAMMATORY CYTOKINES IN VITRO IN A DOSE DEPENDENT MANNER INDICATING THERAPEUTIC POTENTIAL FOR THE TREATMENT OF JIA

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Background: Impaired immunological tolerance in Juvenile Idiopathic Arthritis (JIA) is the result of a disturbed balance between regulatory T-cells (Tregs) and effector T-cells (Teffs). Restoring this balance by either enhancing the suppressive function of Tregs or inhibiting activity of pro-inflammatory Teffs seems a promising therapeutic strategy. We have previously demonstrated that high concentrations of Vitamin B3 (VitB3), also known as nicotinamide (NAM), lead to an increase in FOXP3 positive cells *in vitro*, via suppression of the deacetylase SIRT1. Because the transcription factor FOXP3 is essential for Treg function, this approach could promote immunological tolerance. We now aim to translate these laboratory findings into clinical practice and envision a role for VitB3 as a novel therapeutic strategy for patients with JIA. However, while VitB3 has promising effects on Tregs, the effect of VitB3 on Teff cells, the other side of the scale, is still unexplored.

Objectives: To investigate the effect of VitB3/SIRT1 inhibition on proliferation, activation, cytokine production and TCR signaling of Teff cells.

Methods: T-cells were isolated from the blood of healthy controls and JIA patients, as well as from the synovial fluid from JIA patients. Cells were stimulated with aCD3/aCD28 and cultured in the presence of increasing concentrations of VitB3 (0-9mM) for 1-4 days. Proliferation and expression of activation makers in primary T cells were determined using flow cytometry. Cytokine production was determined by qPCR, Luminex and flow cytometry. ERK phosphorylation was measured by flow cytometry and Western blot. ERK phosphorylation and GFP expression were also determined by flow cytometry using a murine NFAT-GFP reporter cell line.

Results: *In vitro* VitB3 treatment of JIA patient CD4+ Teff-cells significantly decreased the production of the pro-inflammatory cytokines IL-2 and IFN γ measured both on mRNA and protein level. Correspondingly, surface activation markers were downregulated after VitB3 incubation, and ERK phosphorylation was decreased. Furthermore, proliferation of both CD4+ and CD8+ T-cells was inhibited with VitB3 treatment in a dose dependent manner. Murine reporter cells showed similar results. Experimental outcomes were verified using another SIRT1 inhibitor; EX-527.

Conclusion: In addition to the previously demonstrated increase of Treg numbers and functioning, this data demonstrates that VitB3 treatment also inhibits proliferation and activation of Teff cells *in vitro*. VitB3 treatment could therefore modulate the immunological balance by both increasing tolerance and suppressing immune activation. We envision that VitB3 treatment as an adjuvant therapy has the potential to benefit JIA patients and potentially patients with other autoimmune diseases. To assess the clinical relevance of these findings, we are currently in the preparation of a phase III clinical trial.

Disclosure of Interests: None declared

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SAT0032

ACTIVATION OF TH LYMPHOCYTES ALTERS THE PATTERN EXPRESSION AND CELLULAR LOCATION OF VIP RECEPTORS IN HEALTHY DONORS AND EARLY ARTHRITIS PATIENTS

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Background: CD4⁺ T helper (Th) cells are decisive in the struggle against pathogens and in the maintenance of immune homeostasis. Their activation and differentiation can take many forms and generate immune memory in which the extracellular environment plays a critical role in both homeostatic and pathological states. VIP is one of the best-studied neuropeptides with anti-inflammatory and immunomodulatory actions in normal and pathological conditions (1,2,3). There are evidences that VIP

may have therapeutic effects for human Rheumatoid arthritis and might be used as a prognostic marker for this disease (3,4). However, there are no deepen studies related to the expression pattern, cellular location, signalling pathways and functionality of its receptors (VPAC₁ and VPAC₂) during the activation of Th cells. Further in-depth studies of their behavior can contribute to the design of new therapies based on their activation and/or blockade.

Objectives: To describe the expression pattern, cellular location and functional role of VIP receptors, VPAC₁ and VPAC₂, during the activation of human memory Th cells in healthy conditions and in early arthritis (EA).

Methods: Human memory Th cells were isolated from healthy donors (HD) and EA patients. Two experimental conditions, resting state and *in vitro* activation, were used. Cells were activated (anti-CD3/CD28) and cultured in presence or absence of VIP or specific VPAC receptors agonists. Cellular location of VPAC receptors was examined by western-blot and confocal microscopy, and their mRNA levels were analyzed by semi-qPCR. Cytokines, transcription factors and cell markers related to pathogenic profile of Th cells were analyzed by semi-qPCR, ELISA and flow cytometry. To study the signalling pathways, pull-down assays (Rap1), western-blot (CREB) and ELISA (cAMP) were performed. Expression of different chemokines were analyzed by flow cytometry whereas cell migration assays were performed using transwell membranes.

Results: The protein expression pattern of VPAC₁ did not change with the activation of Th lymphocytes, whereas VPAC₂ was up-regulated. In resting cells, VPAC₁ receptor was located on the plasma membrane and nucleus, while it only appeared in the nucleus in activated cells. VPAC₂ was always found in plasma membrane location. VIP receptors signalled through a PKA-dependent pathway in both conditions, and also through a PKA-independent pathway in activated cells. VIP receptors agonists decreased the pathogenic profile during the activation of Th lymphocytes. Similar results were observed in Th lymphocytes from EA patients.

Conclusion: VIP is an important immunomodulator of CD4⁺ T cells in normal and pathological conditions. Our data revealed a change in the expression pattern and cellular location of its receptors (VPAC) during the activation of these cells, giving a wider range of possibilities to dissect its cellular and molecular mechanisms, that would provide new therapies or targets as activity markers in inflammatory/autoimmune diseases.

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SAT0033

TCR REPERTOIRE PROFILING OF SYNOVIAL FLUID OF HLA-B*27+ AND HLA-B*27- PATIENTS WITH PSORIATIC ARTHRITIS

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Background: Diagnosis of psoriatic arthritis (PsA) is primarily based on clinical phenotype because of the diversity of the associated features [1]. The heterogeneity likely implies different mechanisms of the disease progression or initiation. Certain HLA alleles have been shown as risk factors for different spondyloarthropathies (SpAs), implying role of T cells in the pathogenesis. The most prominent association have been shown for HLA-B*27 allele with ankylosing spondylitis (AS), giving raise to the arthritogenic peptide theory of the disease initiation. Recently, using deep TCR profiling, new data supporting potential role of CD8+ T cells in AS have been published by our group and others [2,3]. Despite heterogeneous phenotype 24% of patients from combined cohort of AS and PsA fulfill both CASPAR and modified new-york criteria [4]. Also there is known tendency for axial disease in HLA-B*27+ PsA patients

Objectives: Here we for the first time applied deep TCR profiling to study T cell repertoire of inflamed joints of patients with PsA, to characterise the similarity in clonal composition between patients in presence and absence of HLA-B*27