AB0024

IN VITRO CHARACTERIZATION OF CENERIMOD, A POTENT AND SELECTIVE SPHINGOSINE 1-PHOSPHATE RECEPTOR 1 (S1P₁) MODULATOR IN PREVENTING MIGRATION OF NON-ACTIVATED AND ACTIVATED PRIMARY HUMAN B CELLS IN THE PRESENCE OR ABSENCE OF GLUCOCORTICOID

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Background: Cenerimod is a potent, selective, and orally active sphingosine 1-phosphate receptor 1 (S1P₁) modulator that is currently being evaluated in a Phase 2b study in patients with systemic lupus erythematosus (SLE) (NCT03742037). S1P₁ receptor modulators sequester circulating lymphocytes within lymph nodes, thereby reducing pathogenic autoimmune cells (including B lymphocytes) in the blood stream and in inflamed tissues. Extensive clinical experience has become available for the nonselective S1P receptor modulator fingolimod in relapsing forms of multiple sclerosis, supporting this therapeutic concept for the treatment of autoimmune disorders.

Objectives: Although the effect of S1P-receptor modulators in reducing peripheral B cells is well documented^{1,2}, the role of the S1P₁ receptor on this cell type is only incompletely understood. In this study, the mode of action of cenerimod on primary human B cells was investigated in a series of *in vitro* experiments, including S1P₁ receptor cell surface expression and chemotaxis towards S1P. Moreover, S1P₁ expression following B cell activation *in vitro* was studied. As glucocorticoids (GC) are frequently used in the treatment of patients with autoimmune disorders including SLE, the potential influence of GC on the mode of action of cenerimod was evaluated.

Methods: Primary human B lymphocytes from healthy donors were isolated from whole blood. In one set of experiments, cells were treated with different concentrations of cenerimod to measure S1P₁ receptor internalization by flow cytometry. In a second set of experiments, isolated B cells were activated using different stimuli or left untreated. Cells were then analysed for S1P₁ and CD69 cell surface expression and tested in a novel real-time S1P-mediated migration assay. In addition, the effect of physiological concentrations of GCs (prednisolone and prednisone) on cenerimod activity in preventing S1P mediated migration was tested.

Results: *In vitro*, cenerimod led to a dose-dependent internalization of the S1P₁ receptor on primary human B lymphocytes. Cenerimod also blocked migration of nonactivated and activated B lymphocytes towards S1P in a concentration-dependent manner, which is in line with the retention of lymphocytes in the lymph node and the reduction of circulating lymphocytes observed in the clinical setting. Upon B cell activation, which was monitored by CD69 upregulation, a simultaneous downregulation of S1P₁ expression was detected, leading to less efficient S1P-directed cell migration. Importantly, physiological concentrations of GC did not affect the inhibitory activity of cenerimod on B cell migration.

Conclusion: These results show that cenerimod, by modulating S1P₁, blocks B lymphocyte migration towards its natural chemoattractant S1P and demonstrate compatibility of cenerimod with GC. These results are consistent with results of comparable experiments done previously using primary human T lymphocytes.

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AB0025

CITRULLINATED-PEPTIDE SPECIFIC CD4+ T CELL RESPONSES IN RHEUMATOID ARTHRITIS

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Background: CD4+ T cells reacting to post-translationally modified, citrullinated self-antigens are thought to play a central role in the pathogenesis of rheumatoid arthritis (RA)¹. This is evidenced by a strong HLA class II association, the success of therapeutic co-stimulation blockade and the detection of autoantigen specific T-cells using HLA class II multimers². These cells may represent a target for antigen-specific, tolerogenic therapies and their in-depth phenotyping may provide the means by which to monitor such treatment.

Objectives: To identify the citrullinated-peptide (cit-peptide) induced cytokine repertoire of antigen-specific memory CD4+ T cells in both healthy controls (HCs) and ACPA positive RA patients using intracellular cytokine staining and flow cytometry. Of note, the HLA-types of both HCs and RA patients were not known.

Methods: Cryopreserved peripheral blood mononuclear cells (PBMC) from both HCs (n = 8) and RA patients (n = 13) with both early (untreated) and established disease were thawed and labelled with a proliferation tracking dye (PTD). Labelled PBMC were then either incubated alone or with a pool of cit-peptides for 9-days, followed by a 5-hour restimulation with PMA and ionomycin, where cytokine secretion was blocked for the final 4-hours using brefeldin-A. Cells were then harvested, permeabilised and stained for T cell surface markers and intracellular cytokines including IFN- γ , IL-4, IL-21 and IL-17. Stained cells were

immediately acquired using a BD Fortessa X20, where antigen-specific CD4+ T cells were identified as the viable CD45RO+ (memory) CD4+ T cell population that had proliferated (PTDlow) in response to the cit-peptides. Stimulation indices (SI) were calculated as the percentage of proliferated memory CD4+ T cells in the stimulated wells divided by the percentage in the unstimulated conditions, and cit-peptide responders were defined as those with an SI > 2.0. Net cytokine production was measured by subtracting the percentage cytokine production from unstimulated CD4+ CD45RO+ PTDlow cells, from those stimulated with the cit-peptides.

Results: Comparable proliferative responses were observed in both donor groups in response to stimulation with the cit-peptide pool, where 37 % of HCs and 31 % of RA patients responded with an SI > 2.0 (Fig. 1A). While little cytokine production was observed in the cit-peptide responding HC T cells, for responding RA donors, cit-peptide responsive CD4+ memory T cells were predominantly IFN- γ and IL-21 producing (Fig. 1B and 1C). In contrast, these donors did not produce significant levels of either IL-17 or IL-4 (Fig. 1D and 1E).

Conclusion: Cit-peptides were able to induce proliferation in both HCs and RA memory CD4+ T cells which, amongst the RA donors only, were of a Th1/Tfh subtype. In contrast, and while based only on a small sample, cit-peptides did not induce either IL-17 or IL-4 production in either donor group, suggesting a lack of Th17/Th2 responses. Not all donors responded to the peptide pool, possibly reflecting the limited number of pooled cit-peptides or to a lack of confirmed HLA-DRB1*04:01 positive donors, as peptides were selected for their specificity on this basis. Future work will therefore include HLA-typing, as well as the inclusion of additional citrullinated-epitopes to demonstrate autoreactivity in a wider cross-section of patients. Further phenotyping of the cit-peptide specific T cells will be performed, and future plans will be to study the assay data alongside clinical outcomes to assess its value for immune monitoring.

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Figure 1

Figure 1. Citrullinated-peptide specific memory CD4+ T cell proliferation (A) and net cytokine production of IFN- γ (B), IL-21 (C), IL-17 (D) and IL-4 (E) positive cells.

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AB0026

DECREASE OF ANGIOGENIC T CELLS IN CONNECTIVE TISSUE DISEASE-ASSOCIATED INTERSTITIAL LUNG DISEASE

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Background: Interstitial lung disease (ILD) is one of the most significant complications of connective tissue diseases (CTD), leading to an increase of the morbidity and mortality in patients with CTD [1]. A specific T cell subset termed angiogenic T cells (TAng), that promote endothelial repair and revascularization, have been involved in the pathogenesis of CTD [2-4]. However, to the best of our knowledge, no information regarding the role of TAng in CTD-ILD* is available.

Objectives: To study, for the first time, the potential role of TAng related to vascular damage in CTD-ILD*.

Methods: Peripheral venous blood was collected from 40 patients with CTD-ILD* and three comparative groups: 44 CTD-ILD* patients, 21 idiopathic pulmonary fibrosis (IPF) patients and 20 healthy controls (HC). All subjects were recruited from the Rheumatology and Pneumology departments of Hospital Universitario Marqués de Valdecilla, Santander, Spain. Quantification of TAng was performed by flow cytometry. TAng were considered as triple-positive for CD3, CD31 and CXCR4.

Results: Patients with CTD-ILD* exhibited a significantly lower TAng frequency than CTD-ILD* patients ($p < 0.001$). Similar results were obtained when patients with CTD-ILD* were compared with HC ($p = 0.004$) although no difference was observed between CTD-ILD* and IPF. In addition, a significant increase of TAng frequency was shown in patients with CTD-ILD* in relation to IPF patients ($p < 0.001$), while no difference was observed between CTD-ILD* and HC.

Conclusion: Our results reveal a decrease of TAng frequency related to vascular damage in CTD-ILD*. Furthermore, we disclose that the presence of ILD is associated with lower TAng frequency.

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AB0027

INCREASED CIRCULATING CD39+FOXP3+CD4+ TREG CELLS IN EARLY RHEUMATOID ARTHRITIS FACILITATE THE ANTIINFLAMMATORY ACTION OF METHOTREXATE

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Background: Methotrexate (MTX) remains the first line of treatment in Rheumatoid Arthritis (RA)^{1,2}. Inhibition of AICAR transformylase by MTX results in augmented release of adenine nucleotides to the extracellular space¹; these are rapidly hydrolysed by the combined action of ectonucleotidases CD39 and CD79 rendering the antiinflammatory agent adenosine¹. CD39, the rate-limiting enzyme in this cascade, is highly expressed by a subset of human FoxP3+CD4+ regulatory T cells (Treg39+)²⁻⁴ and MTX may act synergistically with Tregs in the control of inflammation.

Objectives: To study the expression of CD39 on circulating Treg cells of untreated early Rheumatoid Arthritis (ERA) patients and its relation with the ex vivo effect of MTX.

Methods: Peripheral blood was drawn from 22 DMARD- and steroid- naïve ERA patients with a disease duration < 24 weeks, 15 longstanding RA patients (LRA,

disease duration > 2 years) and 37 age and gender-matched healthy controls (HC). LRA patients were receiving low-dose weekly MTX and were naïve to biologicals. 10 ERA patients who had achieved remission 12 months after initiating MTX donated blood for a second time (ERA-R). The frequency of Treg and Treg cell subsets was assessed by flow cytometry. CD4+CD25+CD127- (total T reg), CD4+CD27+CD127-CD39+ Treg (Treg39+) and CD4+CD25-CD39- responder T (Tresp39-) cells were isolated by Ficol-Hypaque, followed by sorting. The suppressor potency of Tregs was assessed in cocultures of isolated Tregs with Tresp, established at different Treg/Tresp ratios. Proliferation was determined by CFSE dilution; cytokine secretion was measured by ELISA of culture supernatants.

Results: As previously described⁵, ERA but not LRA patients demonstrated a superior frequency of circulating Treg (CD4+CD25+CD127-FoxP3+) cells. In addition, the proportion of Tregs that expressed CD39 (Treg39+) was significantly increased in ERA but not LRA. Total ERA Tregs were significantly more potent suppressors of proliferation, TNF α and IFN γ secretion when compared with HC or LRA Tregs, and this difference was partially and significantly abrogated in the presence of adenosine deaminase, or the adenosine A2A receptor (A2AR) antagonists DMPX (3,7-dimethyl-1-propargylxanthine) or ZM 241385, but not in the presence of the adenosine A1 receptor antagonist DPCPX (8-cyclopentyl-dipropylxanthine). When MTX was added to the culture medium, the suppressor potency of total Tregs was further enhanced in all 3 groups of patients, and this enhancement was significantly higher in ERA total Treg/Tresp39- cocultures as compared with HC or LRA total Treg/Tresp39- cocultures. The effect of MTX was also partially and significantly abrogated by adenosine deaminase, DMPX or ZM 241385 but not by DPCPX. We then tested the suppressor potency of isolated Treg39+ together with the enhancer effect of MTX on this potency, and observed that there were no longer differences among ERA, LRA and HC; this further suggests that the differences observed in assays using total Tregs can be attributable to the increased Treg39+ proportions present in ERA. The frequency and function of ERA-R Treg cells were not different from HC or LRA Tregs.

Conclusion: The suppressor action of CD39+Tregs is mediated at least in part by adenosine through A2AR ligation, and the superior suppressive potency of total ERA Tregs is associated with their higher proportion of TregCD39+ cells as compared with HC or LRA. In addition, the augmented suppressor effect observed in the presence of MTX is partly mediated by an increased adenosine production acting on A2AR and is more marked in ERA patients reflecting again their higher proportion of Treg39+ cells. This indicates that MTX cooperates with Treg39+ cells in the control of inflammation.

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AB0028

EXPRESSION OF FATTY ACID TRANSPORTERS AND FREE FATTY ACID UPTAKE IN CD8+ T CELLS IN AUTOIMMUNE ARTHRITIS PATIENTS

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Background: RA CD8+ T cells (CD8s) have been shown to vastly contribute to tissue damage and RA disease activity by presenting and maintaining an active effector phenotype promoting autoinflammation. In order to meet their high metabolic requirements RA CD8s alter not only their glycolytic and glutaminolytic profiles, but also rely on increased lipid metabolism characterized by increased free fatty acid (FFA) uptake and lipid biosynthesis.

Objectives: In order to classify the importance of lipid metabolism in RA CD8s in terms of initiation and perpetuation of effector functions and therefore exploring potential novel therapy targeting points or biomarkers additional investigations have to be made. By analysing FFA uptake and the expression of free fatty acid transporters (FAT) upon TCR-stimulation in RA, PsA, SpA patients and healthy controls (HC), we aimed to further characterize the importance of RA CD8s' lipid metabolism.

Methods: Blood samples from 23 RA patients, 22 SpA patients, 20 PsA patients and 7 HC recruited at Heidelberg University Hospital were processed via negative magnetic separation selection to obtain purified CD8s. CD8s were cultured for 72 hours in [1,6-¹³C] glucose containing medium in the presence of anti-CD3 and anti-CD28. FFA uptake was measured by Palmitate-Bodipy 488 staining (ThermoFisher), the expression of FAT CD36, FABP4 and GPR84 besides CD69, CD36, CD45RA, CD3 and CD8 were assessed by FACS analysis.