**AB0025**

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


**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). This is evidenced by a strong HLA class II association, the success of therapeutically co-stimulation blockade and the detection of autoantigenic specific T-cells using HLA class II multimers. 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) were used. Cells 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/Th17 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.

**References:**


**Disclosure of Interests:** None declared.

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