patients, with a maximum stimulation index of 52.4 compared to a maximum of 6.75 in the HC group. Only 1 HC sample responded with an SI greater than 3.0, whereas 50 % of RA patients elicited responses above this. Two of the RA patients failed to respond to the peptide pool (SI < 1.0). Of note, RA patients were not selected on the basis of tissue type whereas selected peptides bind preferentially to class II HLA containing the shared epitope (SE).

Conclusion: In non-HLA typed individuals, cit-peptide induced proliferative T cell responses were detectable in both RA patients and HCs, and although SIs overall were higher amongst RA patients this did not reach statistical significance in this small sample. Not all RA patients responded to the peptide pool which may be due to the limited number of citrullinated epitopes used, or to RA patients with a non-SE HLA type. Additional work should establish the need for HLA typing in this assay; around half of seropositive RA patients would be expected to be SE positive³. Furthermore, a wider array of cit-peptides may be needed to demonstrate autoreactivity in a broader cross-section of RA patients. Our future plans are to further phenotype the cellular subsets responding to the peptide pool and to study assay data in the context of clinical outcomes, to assess its utility for immune monitoring.

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OP0130

IN VITRO CHARACTERIZATION OF INFLAMMATORY ARTHRITIS ASSOCIATED WITH IMMUNE CHECK POINT INHIBITION

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Background: During cancer treatment with immune checkpoint inhibitors (ICI) such as the anti-PD-1 antibody pembrolizumab, 2-4% of patients develop inflammatory arthritis as an immune related adverse event and half of patients with pre-existing inflammatory arthritis have disease flares. This type of adverse events shows striking similarities with traditional immune mediated inflammatory arthritis. However, the underlying immunological mechanisms of inflammatory arthritis associated with ICI are not fully understood.

Objectives: We aimed to develop an *in vitro* model of inflammatory arthritis associated with ICI, and to use this model to investigate monocyte differentiation and activation following treatment with pembrolizumab.

Methods: First, synovial fluid mononuclear cells (SFMCs) and peripheral blood mononuclear cells (PBMCs) from patients with immune mediated inflammatory arthritis (rheumatoid arthritis and peripheral spondyloarthritis, n=22) and PBMCs from healthy controls were incubated with pembrolizumab and assessed for monocyte chemoattractant protein 1 (MCP-1) secretion by ELISA. Then, cytokine production in SFMCs was studied in more detail by the multiplex V-PLEX proinflammatory panel and by intracellular flow cytometry. Finally, pembrolizumab treated SFMCs were incubated with the different disease modifying anti-rheumatic drugs adalimumab, tocilizumab, tofacitinib, and baricitinib.

Results: Pembrolizumab significantly increased MCP-1 production in the SFMCcultures (P=0.0031). In contrast, pembrolizumab did not change MCP-1 production by PBMCs from neither patients nor healthy controls (P=0.77 and P=0.43). Pembrolizumab also increased the production of TNFα (P=0.049), IFNγ (P=0.047), and IL-12p70 (P=0.031), but did not change the production of IL-6 (P=0.98). Among SFMCs treated with pembrolizumab there was an increased frequency of intermediate monocytes (P=0.044). Interestingly, pembrolizumab also increased the MCP-1 production within the intermediate monocytes only (P=0.028). In contrast, among SFMCs treated with LPS, only the classical monocyte subset was increased (P=0.0045) and MCP-1 production increased in both intermediate and classical monocyte subsets. Lastly, the TNFα inhibitor adalimumab and the Janus kinase inhibitors baricitinib and tofacitinib attenuated the pembrolizumab-induced MCP-1 production (P=0.0004, P=0.033, and P=0.025, respectively) while this was not seen with the IL-6 inhibitor tocilizumab (P=0.75).

Conclusion: We have developed a very simple *in vitro* model of inflammatory arthritis associated with ICI. Using this model, we found that pembrolizumab specifically activated intermediate monocytes and induced TNFα, IFNγ, and IL-12p70 production, whereas IL-6 was unchanged. These findings were supported by effectful reduction of MCP-1 secretion with TNFα inhibition but not with IL-6 inhibition. This model could potentially be used to further study the effects

of ICIs and the underlying immunological mechanisms of inflammatory arthritis associated with ICI.

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OP0131

GUT DERIVED ACETATE PROMOTES REGULATORY B CELLS WITH ANTI-INFLAMMATORY EFFECTS

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Background: Regulatory B cells (Bregs) are defective in many auto-immune diseases, i.e. rheumatoid arthritis (RA). The short-chain fatty acid (SCFA) acetate, derived mostly from gut microbial fermentation of dietary fiber, promotes anti-inflammatory regulatory T cells and protects mice from type 1 diabetes and colitis. We hypothesized that acetate could be a good candidate to promote Bregs in auto-immune diseases.

Objectives: To assess the effect of acetate on Breg number and function, *in vitro* and *in vivo* in mice and humans.

Methods: Bregs were defined as IL-10 producing regulatory B cells (B10 cells). Their number was assessed after overnight exposure to acetate (Ac 10mM) and 4 hours of CpG, ionomycin and PMA in mice and after 24 hours of acetate +/- CpG and 4 hours of ionomycin and PMA in humans. Acetate was given to mice either intraperitoneally (twice at a 12-hour interval) or in drinking water for 3 weeks. Acetate-treated B cells were transferred to mice with collagen-antibody-induced arthritis to assess their function. To decipher the mechanisms behind the effect of acetate, we used inhibitors of GPR43 (CATPB), ATP synthase (oligomycin), glycolysis (2-DG), ACS2 and ACLY and assessed protein lysine acetylation by flow cytometry on human B cells. Acetate and B10 cells were also assessed before and after a 7-day high-fibre diet in 12 healthy volunteers.

Results: In mice, acetate promoted B10 cell differentiation both *in vitro* (medians [IQR] 3.1 [0.4-3.7] and 9.9 [5.9-17.6]% of B for CpG and CpG+Ac respectively, p=0.002) and *in vivo* when intraperitoneal injected [22 [14-29] and 31 [25-37]% of B for PBS and acetate respectively, p=0.03] or added to drinking water [17 [6-25] and 39 [26-40]% of B for water or acetate respectively, p=0.02). Adoptive transfer of acetate-treated B cells protected mice from arthritis compared to non-exposed B cells (ANOVA p=0.008). Acetate also promoted B10 cells from human blood cells (2.5 [1.6-2.7] and 3.4 [2.6-4.5] for unstimulated [Un] and Ac respectively, p=0.0001). Conversely to CpG, acetate specifically promoted IL-10, with no impact or a decrease of proinflammatory cytokines (IL-6: 17 [5-29]; 12 [3-21] and 40 [20-47]% B cells for Un, Ac and CpG respectively, p<0.01 for all comparisons and TNF-α: 48 [29-61]; 41 [28-67] and 69 [64-78]% B cells for Un, Ac and CpG respectively, p<0.01 for CpG vs Un or Ac, NS for acetate vs Un). Inhibition of GPR43 and ACLY did not impact acetate response, while inhibition of glycolysis significantly decreased its effect. Blockade of ACS2, converting acetate into acetyl-CoA, decreased acetate-induced B10 cells. Acetate was associated with an increase of protein lysine acetylation which was not observed in presence of CpG alone, suggesting a different mechanism of action (2.0 [1.3-3.4]; 3.3 [2.4-5.4] and 1.4 [0.5-1.7]% B cells for Un, Ac and CpG respectively, p=0.002 for Un vs Ac, NS with CpG). Conversion of acetate into acetyl-CoA could thus be used for the acetylation of cytoplasmic protein, a post-translational modification that regulates key cellular processes, including energy metabolism. In addition, B10 cells had significantly more lysine-acetylated proteins than IL-10^{neg} B cells or TNF⁺ B cells (5.3 [3.9-7.3]; 3.2 [2.4-5.4] and 3.9 [2.7-6.2] % of B for B10, IL-10^{neg} B cells or TNF⁺ B cells respectively, p<0.01 for all comparisons). Finally, dietary fiber supplementation in healthy individuals was associated with increased acetate and B10 cells in the blood, which were significantly correlated (R²=0.20, p=0.02).

Conclusion: Our results suggest that acetate induces functional Bregs, through its conversion into acetyl-CoA, used for cell metabolism and protein acetylation. Delivery of acetate or acetate producing diets or bacteria might be a promising approach to restore Bregs in non-communicable diseases such as RA in which they are defective.

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