Conclusion: his study showed that survivin binds to DNA and regulates the core gene expression contributing to maturation and function of the IFN γ producing Th1 cells.

References: -

Disclosure of Interests: Malin Erlandsson: None declared, Karin ME Andersson: None declared, Nisha Nair: None declared, Anastasios Damdimopoulos: None declared, Sofia Töyrä Silfverswärd: None declared, Rille Pullerits: None declared, Anne Barton Consultant of: AbbVie, Maria I Bokarewa: None declared

DOI: 10.1136/annrheumdis-2020-eular.4325

OP0128

DISTINCT FEATURES OF HLA-DR+ AND HLA-DR-PD-1HI CXCR5- T PERIPHERAL HELPER CELLS IN SEROPOSITIVE RHEUMATOID ARTHRITIS

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Background: PD-1hi CXCR5- T peripheral helper (Tph) cells are newly identified pathogenic CD4+ helper T cells in rheumatoid arthritis (RA). Since Tph cells have been emerged quite recently, the characteristics of Tph cells as a biomarker of RA are not fully understood.

Objectives: The aim of the study is to evaluate how useful Tph cells in peripheral bloods are compared to other immune cell subsets, and to clarify which Tph subset most accurately reflects the disease activity of RA.

Methods: The RA patients who visited our rheumatology department between January 2000 and February 2017, and met the 2010 ACR/European League Against Rheumatism (EULAR) classification criteria were included. We first assessed correlation with 40 immune cell subsets and the disease activity of RA. Next, the proportions of these immune cells were compared between RA and healthy controls (HCs). We also investigated the immune cell subsets which reflected the time course change of the disease activity after the methotrexate (MTX) treatment. The study protocol was approved by the ethics committee at Keio University School of Medicine.

Results: Thirty-four seropositive RA, 12 seronegative RA and 34 HCs were included. The immune cell subsets which showed correlation with DAS28-ESR ($r > 0.2$ or $r < -0.2$) were activated CD4 T cells ($r = 0.31$), HLA-DR+Th1 cells ($r = 0.20$), HLA-DR+Th1-17 cells ($r = 0.25$), Tfh1-17 cells ($r = -0.25$), HLA-DR+Tph cells ($r = 0.22$), CD3+CD8+naïve T cells ($r = -0.25$), CD3+CD8+effector memory T cells ($r = -0.26$), plasma cells ($r = 0.40$) and CD14++CD16+intermediate monocyte ($r = 0.23$). The proportions of HLA-DR+Th1 cells (2.3% vs. 5.7%), HLA-DR+Th1-17 cells (0.7% vs. 2.2%), Tfh1-17 cells (1.7% vs. 2.0%), HLA-DR+Tph cells (0.02% vs. 0.1%), CD3+CD8+effector memory T cells (16.6% vs. 25.7%), plasma cells (0.04% vs. 0.17%) were statistically higher in the patients with RA compared to HCs. While the proportion of Tph cells showed weak correlation with DAS28-ESR ($r = 0.18$), that was extremely higher in RA (0.08% vs. 0.25%). Interestingly, when assessing the correlations with the disease activity in seropositive and seronegative RA separately, the proportions of Tph cells ($r = 0.52$) and HLA-DR+Tph cells ($r = 0.50$) were highly reflected in seropositive RA, but not in seronegative RA. Regarding the disease activity after the MTX treatment, the change of proportion of Tph cells between week 0 and 52 significantly reflected the change of DAS28-ESR ($r = 0.75$, $p = 0.025$), but not HLA-DR+Tph cells because of the non-specific reduction by the MTX treatment. Rather, HLA-DR-Tph cells significantly reflected the change of DAS28-ESR while receiving the MTX treatment ($r = 0.76$, $p = 0.021$).

Conclusion: Tph cells and HLA-DR+Tph cells highly reflected the disease activity of seropositive RA. However, after the treatment, the proportion of HLA-DR+Tph cells decreased independent from the disease activity, and that of HLA-DR-Tph cells more accurately reflected the change of the disease activity during the treatment.

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Disclosure of Interests: Hiroki Yamada: None declared, Takanori Sasaki: None declared, Katsuya Suzuki: None declared, Masaru Takeshita: None declared, Shuhei Tanemura Employee of: I am employed by Mitsubishi Tanabe Pharma Corporation, Noriyasu Seki Employee of: I am employed by Mitsubishi Tanabe Pharma Corporation, Hideto Tsujimoto Employee of: I am employed by Mitsubishi Tanabe Pharma Corporation, Tsutomu Takeuchi Grant/research support from: Eisai Co., Ltd, Astellas Pharma Inc., AbbVie GK, Asahi Kasei Pharma Corporation, Nippon Kayaku Co., Ltd, Takeda Pharmaceutical Company Ltd, UCB Pharma, Shionogi & Co., Ltd., Mitsubishi-Tanabe Pharma Corp., Daiichi Sankyo Co., Ltd., Chugai Pharmaceutical Co. Ltd., Consultant of: Chugai Pharmaceutical Co Ltd, Astellas Pharma Inc., Eli Lilly Japan KK, Speakers bureau: AbbVie GK,

Eisai Co., Ltd, Mitsubishi-Tanabe Pharma Corporation, Chugai Pharmaceutical Co Ltd, Bristol-Myers Squibb Company, AYUMI Pharmaceutical Corp., Eisai Co., Ltd, Daiichi Sankyo Co., Ltd., Gilead Sciences, Inc., Novartis Pharma K.K., Pfizer Japan Inc., Sanofi K.K., Dainippon Sumitomo Co., Ltd.

DOI: 10.1136/annrheumdis-2020-eular.5552

OP0129

AUTOREACTIVE, CITRULLINATED PEPTIDE-SPECIFIC T CELLS IN RHEUMATOID ARTHRITIS

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Background: Self-reactive CD4+ T cells are thought to play a key role in the pathogenesis of rheumatoid arthritis (RA) and represent a target for antigen-specific, tolerogenic therapies¹. Phenotyping such cells could provide the means with which to monitor these treatments. Here, we aimed to demonstrate antigen-induced T cell responses to RA relevant epitopes using peptide stimulation of peripheral blood mononuclear cells (PBMC). Several autoantigens have been described in RA, including various citrullinated peptide (cit-peptide) epitopes²; here we have used a combination of cit-peptides that will be used to load tolerogenic dendritic cells in a forthcoming experimental medicine study (AuToDeCRA II).

Objectives: To detect peripheral blood T cell proliferation in response to candidate autoantigens in RA patients and healthy controls (HCs) using flow cytometry.

Methods: PBMC from RA patients and HCs were obtained following informed consent and were cryopreserved. Prior to use, cells were thawed in medium supplemented with human serum and were labelled with a proliferation tracking dye (PTD). 2×10^5 labelled cells were plated into 96 well plates with a minimum of 7 replicates per condition and were either cultured alone or stimulated with individual peptides at varying concentrations or a peptide pool. On day 9, cells from replicate wells were harvested, pooled and stained for surface markers and viability. Samples were acquired on a BD Fortessa X20 and analysis was performed in FlowJo version 10. A manual gating strategy was used to identify CD45RO+ (memory) CD4+ T cells and amongst this population, activated/proliferated cells were defined as CD25+ PTD low. Stimulation indices (SI) were calculated as the percentage of activated/proliferated cells from stimulated wells divided by the percentage from unstimulated wells.

Results: Ten RA patients (including early and established disease) were recruited alongside seven HCs. Cit-peptides were initially titrated on an individual basis to determine the optimum concentration for use ($n = 2$). We then compared stimulation with either single peptides or a pool ($n = 2$), and found that PBMC stimulated with the pooled cit-peptides had a higher proliferative response. Both single and pooled cit-peptide induced T cell proliferation was observed in both RA and HC samples (Figure 1); the largest responses were seen amongst RA

Figure 1 Antigen-specific CD4+ CD45RO+ responses of citrullinated peptide stimulated PBMC from HCs and RA patients.

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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Disclosure of Interests: Jessica Swift: None declared, James Stanway: None declared, Catharien Hilken: None declared, Amy Anderson: None declared, Arthur Pratt Grant/research support from: Pfizer, GlaxoSmithKlein, John Isaacs Consultant of: AbbVie, Bristol-Myers Squibb, Eli Lilly, Gilead, Janssen, Merck, Pfizer, Roche

DOI: 10.1136/annrheumdis-2020-eular.4687

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.

References: None.

Disclosure of Interests: Anne Sofie Sørensen: None declared, Morten Nørgaard Andersen: None declared, Kristian Juul-Madsen: None declared, Cæcilie Skejv: None declared, Henrik Schmidt: None declared, Thomas Vorup-Jensen: None declared, Tue Wenzel Kragstrup Shareholder of: iBio Tech ApS, Consultant of: Bristol-Myers Squibb, Speakers bureau: TWK has engaged in educational activities talking about immunology in rheumatic diseases receiving speaking fees from Pfizer, Bristol-Myers Squibb, Eli Lilly, Novartis, and UCB.

DOI: 10.1136/annrheumdis-2020-eular.1356

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.

Disclosure of Interests: Claire DAIEN Grant/research support from: from Pfizer, Abbvie, Roche-Chugai, Novartis, Abivax, Sandoz, Consultant of: Abbvie, Abivax, BMS, MSD, Roche-Chugai, Lilly, Novartis, Speakers bureau: Abbvie, Abivax, BMS, MSD, Roche-Chugai, Lilly, Novartis, Jian Tan: None declared, Rachel Audo: None declared, Julie Mielle: None declared, Laurence Macia: None declared

DOI: 10.1136/annrheumdis-2020-eular.4924