Objectives: We aimed to investigate the proportion and phenotype of peripheral blood ABCs in patients suffering from early drug naïve RA.

Methods: Newly presenting patients, naïve to immunomodulatory treatment, were recruited from the Newcastle Early Arthritis Clinic, and followed until diagnoses were confirmed. B-cell subsets in peripheral blood were detected and phenotyped using flow cytometry.

Results: Our work showed increased proportions of ABCs in seropositive RA compared to other inflammatory arthritis controls, highlighting a potential link between autoantibody production and ABCs. Moreover, patients with high disease activity had higher proportions of ABCs in peripheral blood. Interestingly, the FcRL4⁺, the proliferating Ki67⁺ and the T-bet expressing B cells were enriched in the ABC population compared to the other B cell subsets. Furthermore, ABCs expressed high levels of MHC class II and co-stimulatory molecules, as well as the activation marker, CD69.

Conclusions: These data supports a possible pathogenic role of ABCs in RA, potentially via autoantibody and T cell stimulatory ability, but further characterisation of this subset and functional studies are needed.

Disclosure of Interest: None declared

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THU0034 SALMONELLA TYPHI VI IGG AS A MARKER OF IMMUNOSUPPRESSION IN RHEUMATIC DISEASE

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Background: Measurement of vaccine response may be used as a diagnostic tool to aid determination of antibody deficiency. The IgG response to pneumococcal polysaccharide vaccination (PPV) is currently used to assess T cell independent responses, however additional polysaccharide vaccines are under evaluation. In patients with rheumatic diseases (RD) treatment regimens can result in immunosuppression and subsequently secondary immune deficiency (SID).

Objectives: To measure the IgG response to Typhi Vi vaccination (TV) in RD patients presenting with antibody deficiency. To correlate immunosuppression with TV responses, as well as TV responses to clinical presentations, B cells and total IgG. Further, we interpret the responses in combination with the responses to PPV.

Methods: 35 RD patients were referred for immunological evaluation at Hospital Clínico San Carlos, Madrid, Spain. The responses to TV and PPV were measured using commercial human anti-*Salmonella* Typhi Vi IgG and pneumococcal capsular polysaccharide (PCP) IgG ELISAs kits. A TV responder was defined as achieving ≥ 32 IU/mL (lower limit of the normal range), a PCP IgG responder (>50 mg/L). B cells were measured by flow cytometry (responder $\geq 6.6\%$) and total IgG by nephelometric assay (responder ≥ 600 mg/dL). For all measurements (+) indicates a responder and (-) indicates a non-responder.

Results: A greater percentage of TV- patients previously received non-biological treatment (79% vs 43%), specifically steroid treatments (68% vs 28%) and biological treatments (particularly CD20 and TNF alpha targets; 46% vs 14%). At presentation, TV non-responsiveness was associated with a higher frequency of upper respiratory tract infections (75% vs 57%), serious bacterial infections (21% vs 14%) and required slightly higher antibiotic usage (93% vs 71%). Stratification of the RD patients using the response the TV and PPV identified four groups of activity: TV+/PCP+, TV-/PCP+, TV+/PCP- and TV-/PCP-. In the presence of a normal response to PPV, the failure to respond to TV (TV-/PCP+) correlated with a higher frequency of previous non-biological treatment (84% vs 43%), biological treatment (47% vs 14%), steroids treatment (68% vs 29%) and were currently undergoing treatment (84% vs 29%, $p=0.01$) compared those who responded to TV (TV+/PCP+). At presentation TV-/PCP+ patients had a greater incidence of upper respiratory tract infections (74% vs 57%), serious bacterial infections (16% vs 14%) and antibiotic usage (95% vs 71%). Non-responders to both vaccinations (TV-/PCP-) had a higher incidence of serious bacterial infections (25% vs 16%) and pneumonia (50% vs 32%) when compared to the TV+/PCP- group. When correlated with B cell number, 58% of B cells had a concentration of TV antibodies <32 U/mL.

Conclusions: The response to TV correlated with underlying disease treatment and immunological presentation. Assessing the response to two polysaccharide vaccinations, TV and PPV, may provide a greater understanding of the T cell independent pathway and provide more clinical information for the clinician.

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THU0035 A CD8 ALPHA-NEGATIVE SUBSET OF CD4+SLAMF7+ CYTOTOXIC T CELLS IS EXPANDED IN PATIENTS WITH IGG4-RELATED DISEASE AND DECREASES FOLLOWING GLUCOCORTICOID TREATMENT

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Background: IgG4-Related Disease (IgG4-RD) is a fibro-inflammatory disorder characterised by tumefactive lesions, frequent elevation of serum IgG4 levels, and tissue fibrosis.¹ Glucocorticoids represent the treatment of choice to induce IgG4-RD remission but their effect on the cells orchestrating the disease remains unknown.¹ We recently described an unconventional population of clonally expanded CD4⁺SLAMF7⁺ cytotoxic T effector memory (T_{EM}) cells (CD4⁺CTLs) and causally linked it to IgG4-RD in view of their capacity to secrete pro-fibrotic molecules and to infiltrate affected organs.²⁻⁴

Objectives: In order to better clarify the mechanisms of action of glucocorticoids in IgG4-RD and the pathogenic relevance of CD4⁺ CTLs, we herein aim to describe the effects of corticosteroid treatment on CD4⁺ CTLs.

Methods: CD8 α , granzyme A, perforin, and SLAMF7 expression within the effector/memory compartment of CD45RO (T_{EM}) and CD45RA (T_{EMRA}) CD4⁺ T cells was quantified by flow cytometry in 18 active IgG4-RD patients at baseline and after 6 months of glucocorticoid treatment. Eighteen healthy subjects were studied as controls. Next-generation sequencing of the T-cell receptor α and β chain gene was performed on circulating CD4⁺CTLs in patients with IgG4-RD before and after treatment, and in affected tissues.

Results: Circulating CD4⁺ T_{EM} and T_{EMRA} cells were not expanded in IgG4-RD patients compared to healthy controls. CD4⁺SLAMF7⁺ T_{EM} cells (but not T_{EMRA} cells) were significantly increased among IgG4-RD patients. Within CD4⁺SLAMF7⁺ T_{EM} cells, CD8 α ⁺ but not CD8 α ^{low} cells were elevated in IgG4-RD patients. The same dominant clones of CD8 α ⁺CD4⁺SLAMF7⁺ T_{EM} cells found in the peripheral blood were also identified in affected tissue. Both CD8 α ⁺ and CD8 α ^{low} CD4⁺SLAMF7⁺ T_{EM} cells expressed cytolytic molecules. Clonally expanded CD8 α ⁺ but not CD8 α ^{low} CD4⁺SLAMF7⁺ T_{EM} cells decreased following glucocorticoid-induced disease remission.

Conclusions: A subset of CD8 α ⁺CD4⁺SLAMF7⁺ cytotoxic T_{EM} cells is oligoclonally expanded in patients with active IgG4-RD. This population contracts following glucocorticoid-induced remission. Further characterisation of this cell population may provide prognostic information and targets for therapeutic intervention.

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THU0036 ABATACEPT INCREASES REGULATORY B CELL EFFECT ON T CELL PROLIFERATION THROUGH THE PRODUCTION OF IL-10 AND TGF-BETA IN VITRO AND IN RHEUMATOID ARTHRITIS PATIENTS

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Background: Abatacept is a CD152 agonist known to inhibit T cell proliferation but recent data suggest that it could act directly on B cells.¹⁻²

Objectives: To demonstrate the effect of Abatacept (versus IgG control) on regulatory functions of B cells on T cell proliferation in an established *in vitro* co-culture model. To evaluate its role, *in vivo*, by measuring the regulatory functions of B cells from rheumatoid arthritis patients before and after the Abatacept treatment.

Methods: Peripheral and tonsillar T cell and B cell from healthy controls were purified. The biotinylation of Abatacept was used to study its binding on T and B cells by flow cytometry and confocal microscopy. A well-established co-culture model between CpG-stimulated B cells and anti-CD3/anti-CD28 stimulated T cells was set up in which Abatacept or an IgG control was added to evaluate any change in B cell regulatory functions. Activation markers (e.g. CD25, CD69, CD40, CD152) and regulation markers (e.g. FoxP3, TGF- β) were assessed by flow cytometry. Similar analysis was also performed on rheumatoid arthritis patients before and three months after Abatacept therapy. All patients gave their informed consent.

Results: Abatacept increases the inhibition of T cell proliferation by B cells compared to IgG control in the co-culture model ($p=0.03$). Interestingly, alone, Abatacept does not modify T cell proliferation. This can be explained by the increase in IL-10 and TGF- β producing B cells and the CD152 expression. Abatacept is able to bind B cells at day 0 of co-culture and B and T cells at day 4.5 of co-culture. Abatacept has a direct effect on B cells by increasing the CD25 ($p=0.03$) and CD152 expression ($p=0.02$) reflecting a higher activation level. Nevertheless, Abatacept had no direct effect on B cell proliferation. In RA patients, the treatment with Abatacept resulted in an increased regulation of T cell proliferation and this effect is related to a higher percentage of IL-10 secreting B cells 3 months after the therapy ($p=0.03$).

Conclusions: In our *in vitro* and *in vivo* models, Abatacept has a direct effect on B cells leading to an increase capability of regulation of T cell proliferation which directly linked to higher production of IL-10 and TGF β .

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THU0037

LEPTIN ENHANCED THE EXPRESSION OF AUTOANTIBODIES AND INFLAMMATORY CYTOKINES OF B CELLS VIA ACTIVATING ERK1/2 AND JAK/STAT3,5 PATHWAYS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: B lymphocytes derived from patients with systemic lupus erythematosus (SLE) displayed abnormal activation and overexpression of auto-antibodies. It was reported that leptin was elevated in the sera of SLE patients and lupus mice, and blockade of leptin also remarkably improved the disease activity and renal pathology of lupus mice. Therefore, our study is to explore whether leptin in SLE regulates the activation and function of B cells.

Objectives: Our study is to explore whether leptin in SLE regulates the activation and function of B cells.

Methods: The sera and peripheral blood monocyte cells (PBMC) were isolated from healthy controls and SLE patients with Ficoll, then B cells were acquired through magnetic activated cell sorting (MACS). The sera were incubated in 56° for 30 min (complement inactivation). B cells were cultured in normal or SLE serum, with or without recombinant leptin. Anti-leptin antibody or inhibitions of the signalling pathways were added in SLE sera in order to observe the effects of B cells induced by leptin. Cell proliferation was detected by flow cytometry and carboxyfluorescein diacetate succinimide ester (CFSE), and the levels of auto-antibodies and inflammatory cytokines were examined by ELISA. The total and phosphorylated protein was tested with Western Blot analysis.

Results: Leptin R on B cells showed increased levels in SLE patients [(3.33 $\pm$ 1.28)% v.s. (2.76 $\pm$ 1.06)%, $p<0.01$]. Leptin could upregulate the activated markers (CD80, CD86), also increase the proliferation (CFSE) of B cells. Recombinant leptin promotes IgG/IgM production [IgG: leptin 0 (45.50 $\pm$ 3.87)ng/ml vs. leptin 100 (61.24 $\pm$ 3.66)ng/ml, $p<0.01$; IgM: leptin 0 (60.75 $\pm$ 19.60)ng/ml vs. leptin 100 (118.90 $\pm$ 26.16)ng/ml, $p<0.01$], as well as inflammatory cytokines (IL-6, IL-10, TNF- α) secreted by B cells derived from SLE patients. [IL-10: leptin 0 (20.45 $\pm$ 5.17)pg/ml vs. leptin 100 (23.81 $\pm$ 5.45)pg/ml, $p<0.01$; IL-6: leptin 0 (156.10 $\pm$ 32.64)pg/ml vs. leptin 100 (244.20 $\pm$ 54.23)pg/ml, $p<0.05$; TNF- α : leptin 0 (13.33 $\pm$ 2.38)pg/ml vs. leptin 100 (18.85 $\pm$ 2.69)pg/ml, $p<0.001$]. Compared with Nor serum, SLE serum enhanced the levels of IgG/IgM and inflammatory cytokines, such as IL-6, IL-10, TNF- α . Moreover, blockade of leptin could partially reverse these effects. SLE serum or recombinant leptin could activate p38 MAPK, ERK1/2, and Jak/stat3,5 pathways, but not PI3K/Akt or Jak/stat1 pathways. In addition, blocking ERK1/2 and Jak/stat3,5 pathways could partially reverse the effects of enhanced secretion of autoantibodies and inflammatory cytokines induced by recombinant leptin or SLE serum.

Conclusions: Leptin promote the activation and proliferation of B cells in SLE. Recombinant leptin or serum leptin from SLE patients both enhanced the

expression of autoantibodies and inflammatory cytokines (IL-6, IL-10, TNF- α) of B lymphocytes. Additionally, ERK1/2, and Jak/stat3,5 signalling pathways might play a vital role in this process.

Disclosure of Interest: None declared

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THU0038

INCREASE OF CIRCULATING MEMORY B CELLS AFTER GLUCOCORTICOID-INDUCED REMISSION IDENTIFIES PATIENTS AT RISK OF IGG4-RELATED DISEASE RELAPSE

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Background: IgG4-related disease (IgG4-RD) is relapsing-remitting systemic fibro-inflammatory condition characterised by elevated serum IgG4 concentration and by tumor-like lesions^[1]. Glucocorticoids represent the treatment of choice to induce IgG4-RD remission but relapses occur in almost 50% of patients at two years.² Several evidences suggest that B cells are central to the pathogenesis of IgG4-RD, the most significant being (i) the clinical improvement induced by B-cell depletion with rituximab, and (ii) the oligoclonal expansion of circulating plasmablasts in the vast majority of patients.³

Objectives: To describe alterations of B-lymphocyte subpopulations that might predict IgG4-RD relapse in patients treated with a first course of glucocorticoids according to international guidelines

Methods: Thirty patients with IgG4-RD were treated with glucocorticoids according to international consensus guidelines. Flow cytometry analysis of circulating CD19⁺ and CD20⁺ cells, naïve B cells, memory B cells, plasmablasts, and plasma cells was performed at baseline and every 6 months after the initiation of corticosteroid treatment.

Results: Patients with active untreated IgG4-RD showed reduced CD19⁺ B cells, CD20⁺ B cells, and naïve B cells compared to healthy controls ($p<0.05$), but expanded plasmablasts and plasma cells ($p<0.01$). Glucocorticoids treatment led to disease response in all patients. Clinical improvement was accompanied by a significant reduction of naïve B cells, circulating plasmablasts, and plasma cells, and by a significant increase of memory B cells compared to baseline ($p<0.01$). Increase of circulating memory B cells was observed only in patients experiencing disease relapse and not in patients who maintained remission at two years of follow-up (HR:14.40, 95% CI 2.96–70.1, $p<0.01$, figure 1 A-B). Relapse rates at 12 and 24 months were 25% and 100% with memory B cell increase at 6 months (figure 1C), respectively. No B-cell subpopulations were found to predict IgG4-RD relapse at disease onset.

Conclusions: The efficacy of glucocorticoids in IgG4-RD is associated with selective effects on different B-cell subpopulations. IgG4-RD relapse may be predicted by the increase of memory B-cells after glucocorticoid-induced remission

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