peripheral blood of rheumatoid arthritis (RA) patients (pts), but differences between B-cell subsets distributions in early and late onset RA (LORA) are still unclear [1,2].

Objectives: To examine B-cell subsets in peripheral blood of healthy donors, early (disease duration <6 months) RA (ERA) and LORA pts by flow cytometry, and to analyze the association between B-cell subsets and RA activity.

Methods: 24 active ERA pts (20F/4M); median age 54 years (range) (38–64); disease duration 5 months (4–6); DAS28 score 5.2 (4.7–5.9); RF+75%, ACCP+ 87.5% and 20 LORA pts (16F/4M); median age 58 years (range) (44–62); disease duration 12 years (4.5–17.5); DAS28 score 5.3 (4.6–6.2); both RF+ and ACCP+ 80%, were assessed for B-cell subpopulations and laboratory data: ESR, CRP. LORA pts were treated with DMARDs (anti-TNF- α , Methotrexate), glucocorticoids, and NSAIDs; ERA pts received NSAIDs only. CD19+B cells, memory B cells (CD19+CD27-), non-switched memory B cells (CD19+IgD+CD27+), switched memory B cells (CD19+IgD-CD27+), naïve (CD19+IgD+CD27-), double-negative (CD19+IgD-CD27-), transitional (CD19+IgD+CD10+CD38+CD27-) B cells, and plasmablasts (CD19+CD38+++IgD-CD27+CD20-) were analyzed using multicolor flow cytometry.

Results: The median percentages (range) of non-switched memory B cells (CD19+IgD+CD27+) were lower in ERA and LORA RA cohorts compared to donors: 3.0% (1.6–5.5) and 6.2% (2.4–10.2) vs 7.4% (3.7–11.1), respectively, $p < 0.05$ for both cases. In LORA pts, the median percentages (range) and absolute numbers of switched memory B cells (CD19+IgD-CD27-) were higher compared to both ERA pts and donors: 24.1% (14.0–42.3) vs 8.3% (4.4–12.9) and 12.8% (9.3–17.0); and 0.03 (0.02–0.05) vs 0.01 (0.006–0.02) and 0.02 (0.01–0.04), respectively; $p < 0.05$ for all cases. The median percentages of naïve B cells (CD19+IgD+CD27-) were significantly higher in ERA pts compared to LORA pts and healthy donors: 75.3% (69.7–83.6) vs 54.3% (39.7–69.1) and 64.7% (57.6–72.4), respectively, $p < 0.05$ for both cases.

In ERA pts, we found a significant correlation between absolute numbers of non-switched memory B cells (CD19+IgD+CD27+), RF and anti-CCP: $r = 0.50$ and 0.45, respectively, $p < 0.05$ for both cases. In LORA pts, the percentage of memory B cells (CD19+CD27+) correlated positively with ESR ($r = 0.58$), $p < 0.05$; a positive association was observed between the absolute numbers of switched memory B cells (CD19+IgD-CD27+), CRP ($r = 0.48$) and RF ($r = 0.49$), $p < 0.05$ for all cases.

Conclusions: In ERA pts, immunophenotyping showed increased frequencies of naïve B cells and decreased frequencies and absolute numbers of switched memory B cells compared to LORA cohort.

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SAT0025 MIR 106A, MIR 19A-B, MIR 20A AND MIR21A REGULATE V γ 9V δ 2 FUNCTIONS PARTICIPATING IN THE INFLAMMATORY RESPONSES OCCURRING IN RHEUMATOID ARTHRITIS

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Background: miRNAs are non-coding RNAs which have significant roles in regulating gene expression. The miR17-92 cluster appears to be a key factor in the inflammatory pathways activated during RA.

Objectives: In this study we aimed to evaluate miR17–92 expression and functions in $\gamma\delta$ T cell subsets in RA patients, $\gamma\delta$ T cells, in fact produce pro-inflammatory cytokines such as IFN- γ , IL-6 and IL-8 that may contribute to the inflammatory responses in RA.

Methods: Heparinized peripheral blood from 10 early RA untreated patients and 10 healthy donors was obtained for this study. Polyclonal V γ 9V δ 2 T cell lines were generated first by magnetic isolation followed by sorting (FACSARIA) and further analysed by flow cytometry to define their phenotype and their pattern of cytokine production. Expression analysis of miRNA17–92 cluster was performed by RT-PCR and after identification of relevant miRNA involved in RA, mRNA expression of miRNA target genes was studied.

Results: A remarkable change in the distribution of V γ 9V δ 2 T cell functional subsets with an expansion of effector subsets and a reduction of naïve cells, was observed in the peripheral blood of RA patients, as compared to healthy donors, which were accompanied by modifications in proinflammatory cytokine expression. Particularly, T_{EM} (effector memory) and T_{EMRA} (effector memory terminally differentiated) cells resulted the major source of IL-6 and IL-8. A significant correlation between miRNA expression and levels of IL-6 and IL-8 was found. The comparative analysis of miRNA expression among V γ 9V δ 2 T cell subsets, between RA patients and healthy controls showed a downregulation of miR106a-5p and miR20a-5p and an upregulation of miR 21a-5p among V γ 9V δ 2 T_{EM} cells; a downregulation of miR19–3p among V γ 9V δ 2 T_{EM} and T_{CM} (central memory) cells was also found. This miRNA expression regulated IL-8 and IL-6 gene transcription contributing to the

survival of the pro-inflammatory pool reducing the expression of the PDCD4 gene.

Conclusions: Our results provide evidence of a role of miR106a, miR19–3p, 20a and 21a in the regulation of V γ 9V δ 2 T cells function in RA patients and suggest the possibility that the miR17–92 family and $\gamma\delta$ T cells participate to the pathogenesis of RA.

Disclosure of Interest: None declared

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SAT0026 SIGNALLING THROUGH INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR CONTRIBUTES TO IL-6 PRODUCTION AND SUPPORTS T CELL DEPENDENT INFLAMMATION IN RHEUMATOID ARTHRITIS

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Background: Insulin-like growth factor (IGF) 1 receptor is essential for cell energy metabolism. It plays a key role linking glucose and lipid metabolism of active cells and may turn pathogenic in uncontrolled tissue growth in tumours. In rheumatoid arthritis (RA), the IGF-1R signalling is activated supporting expansion of the inflamed synovia.

Objectives: The aim of the present study was to understand the place of IGF-1R expression and signalling for development of adaptive immune responses in the setting of prospective RA cohort and in experimental arthritis.

Methods: Clinical associations of IGF1R expression in leukocytes of the peripheral blood were studied in 84 female RA patients with mean disease duration 8 years, all treated with methotrexate. Consequences of the IGF1R signalling inhibition for arthritis were studied in mBSA immunised Balb/c mice treated with NT157 compound promoting degradation of the insulin receptor substrates IRS-1/2.

Results: In RA patients, high expression of IGF1R in leukocytes was associated with systemic inflammation as verified by higher transcription factor NF- κ B ($p = 0.033$), serum levels of IL-6 ($p = 0.006$) and erythrocyte sedimentation rate ($p = 0.003$), and higher pain perception (VAS, $p = 0.019$). Additionally, women with high IGF1R were often in need of combined DMARD treatment ($p = 0.045$). Phosphorylated IGF1R and STAT3 enrich T cells infiltrate in RA synovia. Treatment with NT157 rendered no metabolic consequences to mouse body weight and serum glucose levels. NT157 inhibited the phosphorylation of IGF1R and STAT3 in synovia, and alleviated arthritis and erosions in mice. It also reduced IGF1R+ T cell population in spleen and despaired ERK and Akt signalling. This limited the ability of mBSA-specific T cells to produce IL-6 ($p = 0.013$), IFN γ ($p = 0.024$) and IL-17 ($p = 0.0097$). The reduction of IL-6 and IL-17 levels was associated with changed RoRgt/FoxP3 balance.

Conclusions: IGF1R signalling contributes to severe RA by triggering T cell dependent inflammation in arthritis. Inhibition of IGF1R on the level of insulin receptor substrates alleviates arthritis by restricting IL-6 dependent formation of Th17 cells and may open for new treatment strategies in RA.

Disclosure of Interest: None declared

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SAT0027 IMMUNOMODULATORY EFFECTS OF MGD010, A DART® MOLECULE TARGETING HUMAN B-CELL CD32B AND CD79B

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Background: B-cell targeted therapeutics have proven efficacious in the treatment of autoimmune diseases, generating impetus to develop alternate strategies that provide activity with rapid onset and no B-cell depletion. To this end, MGD010, a bispecific DART® molecule, was designed to exploit the inhibitory function of the checkpoint molecule, CD32B, (Fc γ RIIB) via its simultaneous co-ligation with the BCR component, CD79B. Initial phase 1 studies performed in healthy subjects demonstrated MGD010 was well tolerated in a single-dose, dose escalation to up to 10 mg/kg, with no evidence of peripheral B-cell activation or depletion. MGD010 exhibited pharmacokinetic properties of an antibody-based molecule, with dose-dependent B-cell occupancy saturating at >1mg/kg and diminished propensity for ex-vivo BCR-induced B-cell activation (1). To further understand the potential for MGD010 to modulate ongoing immune responses, the impact of MGD010 on the immune response to Hepatitis A (HAV) vaccination, a T-cell dependent neo-antigen challenge, was evaluated in normal healthy subjects.

Objectives: To determine effects of MGD010 on humoral and cellular immune responses in normal healthy volunteers.

Methods: This was an open label, placebo controlled cohort-expansion in normal healthy volunteers. Subjects (n=8 per group) were randomly assigned to receive an IV administration of a single dose of 3 or 10 mg/kg MGD010 or placebo. All subjects received a single intramuscular dose of hepatitis A vaccine (HAV) (1.0 mL or ~50U) 24 hours after administration of MGD010 or placebo. Subjects were monitored for adverse events for 56 days after administration of drug or placebo. Analyses included peripheral lymphocyte

counts and immunophenotyping, determination of serum immunoglobulin levels, seroconversion rate of HAV and quantification of serum HAV IgG concentration.

Results: Twenty-four (24) healthy subjects were enrolled for the study and 23 completed the study. One subject discontinued prior to completion due to inability to follow study procedures. There were no severe adverse events in subjects who received 3 or 10 mg/kg MGD010. The only serious adverse event reported in a subject who had received placebo and was not drug related. There were no CTCAE grade 3 or higher adverse events related to MGD010. Consistent with prior observations (1), ex vivo flow cytometric analysis confirmed dose-dependent MGD010 binding to peripheral B cells without B-cell depletion, accompanied with decreased surface BCR and CD40 expression as well as a moderate decrease in total serum IgM levels. Reduced HAV seroconversion rates were observed in subjects treated with MGD010 as compared to placebo, with significantly lower HAV-specific IgG levels in subjects treated with MGD010 compared with placebo group ($P < 0.05$).

Conclusions: These studies demonstrate that by pharmacologically exploiting the activity of the checkpoint molecule CD32B in combination with the BCR CD79B component, a single dose administration of either 3 or 10 mg/kg MGD010 delivers an immunomodulatory effect that effectively counters B-cell function. Together with a good safety profile, these data provide compelling rationale for further developing MGD010 as a therapeutic modality for autoimmune diseases.

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SAT0028 AIOLOS OVEREXPRESSION IN SYSTEMIC LUPUS ERYTHEMATOSUS B-CELL SUBTYPES AND BAFF INDUCED MEMORY B-CELL DIFFERENTIATION ARE REDUCED BY CC-220 MODULATION OF CEREBLON ACTIVITY

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Background: Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by alterations in B-cells, autoantibody production, and elevations in circulating B-cell activating factor (BAFF). Aiolos single nucleotide polymorphisms and elevations in Aiolos mRNA are associated with SLE susceptibility. CC-220 is a Cullin Ring Ligase 4 Cereblon (CRL4CRBN) E3 Ubiquitin Ligase Modulator that induces ubiquitination and degradation of Aiolos and Ikaros transcription factors. We investigated the effects of CC-220 in B-cells from SLE patients to assess its impact on Aiolos protein levels and B-cell proliferation, differentiation, and antibody production.

Objectives: Determine the protein levels of the B-cell transcription factors Aiolos and Ikaros in B-cell subtypes of SLE patients and assess the impact of the cereblon modulator CC-220 on Aiolos and Ikaros protein degradation, proliferation, and plasmablast differentiation.

Methods: Peripheral blood mononuclear cells (PBMC) were measured for levels of circulating B-cell subtypes, B-cell activation state, and Aiolos and Ikaros protein levels by flow cytometry. Cell types were defined as: CD27-IgD⁺ naive, CD27⁺IgD⁻ switched memory, CD27⁺IgD⁺ nonswitched memory, and CD27-IgD⁻ double negative B-cells and CD20⁺CD38⁺ plasmablasts. Measurements of BAFF, IL-2 and IL-21 from plasma and IgG and IgM were done by ELISA. Plasmablast differentiation was induced by BAFF, IL-2, and IL-21 co-stimulation of B-cell cultures for 5 days in the absence and presence of CC-220.

Results: Plasma samples from SLE patients were found to have higher circulating BAFF, similar IL-2 levels, and reduced IL-21 levels relative to healthy individuals. Alterations in circulating B-cell subtypes occur in SLE patients including reduction of memory and elevations of naive and double negative B-cells. Alterations in biomarkers associated with chronic B-cell activation, including elevation of CD95 and reduction of CD21 and CD23 were also observed. Assessment of the B-cell differentiation transcription factors Aiolos and Ikaros in SLE B-cells showed that Aiolos, but not Ikaros is elevated in naive, switched memory, nonswitched memory, and double negative B-cells. CC-220 treatment reduced Aiolos and Ikaros protein levels in both SLE and healthy individuals in all four B-cell subtypes measured. CC-220 treatment was associated with reduced B-cell proliferation, plasmablast differentiation, and secretion of IgG and IgM in cells co-stimulated with BAFF, IL-2 and IL-21. Similarly, CC-220 reduced the expression of genes involved in B-cell differentiation including IRF-4, Xbp-1, Blimp-1, and IgJ indicating an early blockade in B-cell differentiation.

Conclusions: Our observations that SLE is associated with alterations of circulating B-cell subtypes and their activation state, Aiolos overexpression in B-cells, and increases in circulating BAFF support the hypothesis that B-cells play a significant role in SLE pathology. Moreover, these observed changes

suggest that B-cells may be predisposed towards plasmablast differentiation and antibody production in SLE. CC-220 administration reduced Aiolos and Ikaros protein levels in B-cells and reduced proliferation, plasmablast differentiation and antibody production at clinically obtainable drug concentrations (< 10 nM).

Disclosure of Interest: G. Ringheim Shareholder of: Celgene Corporation, Employee of: Celgene Corporation, J. Kosek Shareholder of: Celgene Corporation, Employee of: Celgene Corporation, L. Capone Shareholder of: Celgene Corporation, Employee of: Celgene Corporation, P. Schafer Shareholder of: Celgene Corporation, Employee of: Celgene Corporation, Y. Nakayama Shareholder of: Celgene Corporation, Employee of: Celgene Corporation

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SAT0029 B CELL DEPLETION AFFECTS CD8 T CELLS IN ANCA-ASSOCIATED VASCULITIS

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Background: In anti-neutrophil cytoplasmic antibodies associated vasculitis (AAV), several clues suggest that the efficacy of B cell depletion therapy lies beyond the suppression of ANCA-producing cells and may involve the suppression of B-T cell crosstalk. However, little if any data are available regarding the impact of rituximab on CD4, regulatory T and CD8 T cells in this setting.

Objectives: To compare the effect of conventional immunosuppressants (CIS) and rituximab (RTX) on 3 T cells compartments in AAV. To assess the impact of patients B cells and that of B cell depletion on CD8 T cell cytokine production.

Methods: Thorough cross-sectional immunophenotypic analysis of CD4, regulatory T and CD8 cells of 53 patients with AAV, using polychromatic flow cytometry. A multiplex Luminex immunoassay was used to measure Cytokine/chemokine production of in vitro stimulated CD8 T cells. Active untreated AAV patients' B cells and/or CD8 T cells were cocultures for 72h with Staphylococcal Enterotoxin (Autologous and criss-cross experiments), and CD8 T cells cytokine production was then measured by flow cytometry.

Results: Among CD4 T cells, we found that frequency of naïve and memory subsets and the expression of CCR5, CCR4 and CD161 were not influenced by maintenance treatment type. Similarly, total Treg frequency and Treg subsets including CD161⁺, Helios⁺, resting (CD45RA⁺) and memory (CD45RA-FoxP3^{hi}) Tregs were comparable among RTX and CIS treated patients. By contrast, the type of maintenance treatment markedly influenced the CD8⁺ T cell compartment. Patient under B cell depletion therapy had less TEMRA (CD45RA+CCR7⁻) and more TEM cells than those receiving CIS, and resembled those in long term remission off therapy. CMV seropositivity did not explained the observed differences. Longitudinal data confirmed that B cell depletion significantly decreased TEMRA (CD45RA+CCR7⁻) CD8 T cell frequency ($p < 0.001$), whereas CIS had the opposite effect. Furthermore, we found that in vitro stimulated CD8 T cells from B cell depleted patients produced less pro-inflammatory cytokine/chemokine than those from patients treated with CIS. In coculture with B cells and SEB, patients CD8 cells coculture produced higher level of inflammatory cytokines than those from controls, but only when they were stimulated with patients' B cells.

Conclusions: B cell depletion therapy has a significant impact on the CD8 T cell compartment in terms of phenotype and function. B cell can promote pro-inflammatory function of CD8 T cell in vitro. CD8 T cell are found in vasculitis lesions. These observations raise the question whether the disruption of B cell help to CD8 T cells could contribute to the dramatic efficacy of RTX. Further studies are needed to demonstrate the implication of patients' CD8 T cell in tissue damage.

Disclosure of Interest: None declared

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SAT0030 BREACH OF AUTOREACTIVE B-CELL TOLERANCE BY POST-TRANSLATIONALLY MODIFIED FOREIGN PROTEINS

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Background: Autoimmunity in Rheumatoid arthritis (RA) patients is characterized by a spectrum of anti-modified protein antibodies (AMPA) directed against post-translationally modified (PTM) proteins. The best-known AMPA in RA are anti-citrullinated protein antibodies (ACPA). Much less is known about the occurrence and aetiology of other AMPA responses in RA such as autoantibodies directed to malondialdehyde-acetaldehyde (MAA) adducts, acetylated antigens, and carbamylated proteins. Anti-carbamylated protein (anti-CarP) autoantibodies recognize carbamylated proteins containing a homocitrulline, a PTM structurally similar to citrulline. With the presence of various AMPA responses in RA, PTM proteins have been implicated in the breach of autoreactive B-cell tolerance