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.

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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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THU0039 INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR REGULATES THE PHENOTYPE AND FUNCTION OF CD21+ B CELLS

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Background: Ligand to the inducible T cell costimulator (ICOSL) on B cells is essential for the ICOS-dependent follicular recruitment of activated T cells. In patients with rheumatoid arthritis (RA) the IGF1-IGF1R axis is altered. Inhibition of IGF1R alleviated arthritis by reducing IL-6-dependent formation of Th17 cells.¹ Here we study the role of IGF1R on CD21+ cells in experimental arthritis.

Methods: Female Balb/c mice were immunised with methylated BSA or with CII. Consequences of the IGF-1R inhibition for arthritis were studied in mBSA and CII-immunised mice treated with NT157 compound promoting degradation of insulin receptor substrates or using shRNA producing construct (shIGF1R). At termination three sub-populations of CD21+ cells were analysed: follicular dendritic cells (FDC, CD21+CD19-CXCR5-); marginal zone B cells (MZBc, CD21+CD19+CXCR5-); follicular B cells (FBc, CD21+CD19+CXCR5+). Supernatants of LPS-stimulated splenocytes were analysed for production of cytokines, chemokines using Cytokine Array. Serum levels of antigen specific and autoantibodies were measured in an ELISA.

Results: In spleen of mBSA-immunised mice, ICOSL expression on CD21+ cells correlated to IGF1R ($r=0.70$, $p=0.007$). Inhibition of IGF1R induced a 20% reduction in ICOSL expression in all CD21+ subsets ($p=0.007$) followed by an increase in the number of MZBc ($p=0.003$), while FDC and FBc were unchanged. Inhibition of IGF1R had no effect on the expression of ICOSL on CD4 T cells or the subset of CXCR5+ follicular T cells. Reduction of the ICOSL+CD21+B cells was associated with lower production of IL-13. Inhibition of IGF1R signalling by NT157 and by shRNA, reduced production of CXCL13 and CXCL12, the chemokines essential for B cell migration towards follicles. In contrast, the production of chemokines CCL5 and CXCL12 preventing intra-follicular migration was increased, which explains the increase of MZBc. Additionally, the insufficient ICOSL signalling significantly reduced the production of IL-7 and IL-4, regulating class switching of B cells in germinal centres and differentiation of B cells into plasma cells. The described disbalance in the cytokines aiding B cell development led to the reduced production anti-inflammatory IL-10 and of mBSA-specific IgM ($p=0.005$) and increased production of autoreactive RF-IgM levels ($p=0.001$).

Conclusions: The study shows that IGF1R controls B cell development through the expression of ICOSL on CD21+ cells. Insufficient ICOSL signalling disturbs a balance between antigen-specific response and autoantibody production in experimental arthritis.

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THU0040 ENHANCEMENT OF EARLY HUMAN B CELL DEVELOPMENT BY JAK INHIBITION

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Background: Rheumatoid arthritis is an immune-mediated disease, in which immune cell activation leads to destructive inflammation of the joints. In this context the treatment with JAK inhibitor tofacitinib has been proven to be effective. In mice treatment with tofacitinib resulted in reduced specific antibody responses, failure to generate germinal centres, and partial block of B cell development in the bone marrow. Conversely *in vivo* treatment of psoriasis arthritis patients as well as of rheumatoid arthritis patients with tofacitinib results in an increase of relative

and absolute numbers of B cells in the first 4–8 weeks from beginning of treatment. It is known that mouse early B cell development is strongly dependent on IL-7 signalling, while this is not essential in humans. Nevertheless other cytokines are important in determining the fate and development of B lymphocytes. Hence, the outcome of JAK inhibition in early B cell development remains to be studied

Objectives: To assess the impact of JAK inhibition on early B cell development *in vitro*

Methods: We used a *in vitro* model in which CD34+ cells isolated from cord blood are cultivated subsequently in SCF, Flt3-L and IL-6, then SCF, Flt3-L and IL-7 and finally in cytokine-free medium.¹ The culture reproduces all stages of development from common lymphocytes progenitors to immature B cells.

Results: With the addition of tofacitinib to the *in vitro* culture we observed an increase in the absolute numbers of lymphoid precursors developing *in vitro* especially at week 5 and 6 of culture. Specifically, JAK inhibition led to an increase of pre-B and immature B cells by week 5 and 6. These data are in contrast with the early B cell development block observed in the tofacitinib treated mouse, but are in line with the rapid increase of B cells in peripheral blood after 4–8 weeks of tofacitinib treatment in patients. Analysis of induction of fate determining genes (EBF, E2A, PAX-5) showed an earlier and stronger induction of fate determining genes.

Conclusions: Our data indicate that JAK inhibition may promote early B cell development by enhancing the commitment of lymphoid precursors to the B cell compartment, contributing to a temporary increase in relative and absolute numbers of B cell in peripheral blood of treated patients. These data contribute to our understanding of human B cell development, prompt us to further analyse the quality of B cell output from the bone marrow in JAK inhibited patients, and may provide cues to understand the outcome of JAK inhibition treatment in rheumatic diseases.

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THU0041 THE EFFECTS OF ABATACEPT ON HUMAN B CELL ACTIVITIES

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Background: Abatacept is a cytotoxic T lymphocyte antigen-4 (CTLA-4) fusion protein approved for rheumatoid arthritis (RA) treatment worldwide. Abatacept mimics the natural CTLA-4 and competes with CD28 for binding the CD80/CD86 on antigen presenting cells to prevent T cell activation. However, the impacts of CTLA-4-Ig through its interaction with CD80/CD86 on B cells are not fully understood.

Objectives: The aim of this study was to test whether CTLA-4 regulates human B cell functions

Methods: We assayed the effect of abatacept on human B cells in both *in vitro* and *in vivo* conditions. Blood was taken from 20 patients with RA before and after abatacept treatment and the expression of surface proteins on B cells was detected using immunofluorescent staining. Serum level of rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibody (ACPA) was measured by ELISA. Purified human B cells from healthy donors were stimulated in the presence of abatacept and cell proliferation, cytokine production, plasma cell differentiation, and antibody production were measured.

Results: In RA patients, abatacept treatment transiently reduced the level of CD80/CD86 on peripheral blood memory B cells and increased the naïve to memory B cell ratio. Also, abatacept reduced IgM-RF level in 10 out of 12 patients, which correlates with patients' disease activity, but it had no significant effects on serum levels of anti-citrullinated protein antibody or anti-tetanus toxoid antibodies during the 6 months of abatacept treatment. In the *in vitro* assays, we observed that the CD80 and CD86 induced by T-independent (TI) but not T-dependent (TD) stimulation was significantly downregulated by abatacept at both the mRNA level and protein level. Some TI-induced cytokine production by B cells from healthy donors was also reduced by abatacept. Neither TI nor TD-stimulated B cell proliferation was reduced by abatacept in ³H-thymidine incorporation assay. Abatacept had no significant effect on CD38^{high} CD27^{high} plasma cell differentiation. Finally, abatacept inhibited Daudi-B cell induced allogeneic T cell proliferation, indicating a significant blockade of T-B interaction by abatacept.

Conclusions: In RA patients, abatacept may decrease RF level by interfering the interaction of CD28 with CD80/CD86, therefore preventing B cells from T cells'