Methods: Peripheral and tonsilar 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 were 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 TGFβ.

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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 succinimdyl 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 ±1.28)% v.s. (2.76±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±3.87)ng/ml vs. leptin 100 (61.24±3.66)ng/ml, p<0.01; IgM: leptin 0 (60.75±19.60)ng/ml vs. leptin 100 (118.90±26.16)ng/ml, p<0.01], as well as inflammatory cytokines (IL-6, IL-10, TNF-a) secreted by B cells derived from SLE patients. [IL-10: leptin 0 (20.45±5.17)pg/ml vs. leptin 100 (23.81±5.45)pg/ml, p<0.01; IL-6: leptin 0 (156.10 ±32.64)pg/ml vs. leptin 100 (244.20±54.23)pg/ml, p<0.05; TNF-a: leptin 0 (13.33 ±2.38)pg/ml vs. leptin 100 (18.85±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-a. 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-a) of B lymphocytes. Additionaly, 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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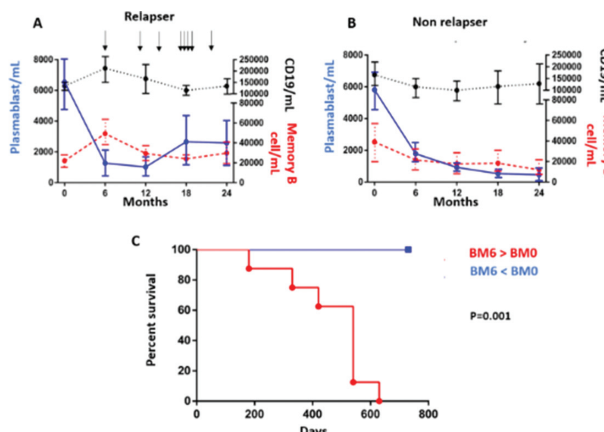
Background: IgG4-related disease (IgG4-RD) is relapsing-remitting systemic fibro-inflammatory condition characterised by elevated serum IgG4 concentration and by tumor-like lesions[.[1] Glucocorticoids represent the treatment of choice to induce IgG4-RD remission but relapses occur in almost 50% of patients 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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