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.

Conclusions: 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 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.

REFERENCES:

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THU0037
LEPTIN ENHANCED THE EXPRESSION OF AUTOANTIBODIES AND INFLAMMATORY CYTOKINES OF B CELLS VIA ACTIVATING ERK1/2 AND JAK/STAT3 PATHWAYS IN SYSTEMIC LUPUS ERTHEMATOUS

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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°C 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 succinimidyl 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]% vs. [2.76±1.06]% , p<0.01). Leptin could upregulate the activated markers (CD90, CD69), also increase the proliferation (CFSE) of B cells. Recombinant leptin promotes IgG1/IgG3 production [ IgG1: leptin 0 (45.5±3.87)ng/ml vs. leptin 100 (61.24±3.66)ng/ml, p<0.01; IgG3: 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-α) 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-α: 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 IgG1 and inflammatory cytokines, such as IL-6, IL-10, TNF-α. Moreover, blockade of leptin could partially reverse these effects. As a result of the incubation with recombinant leptin and/or anti-LEP, the levels of IgG1 and inflammatory cytokines were decreased.

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.