

joint replacement surgery. ASCs were isolated and cultured with/without human recombinant leptin, TNF, adiponectin (LMW, HMW) and IFN γ . After 24 h, secretion of cytokines was measured by ELISA. Conditioned media from ASCs cultures were used to stimulate FLS. After stimulation, FLS proliferation and apoptosis was determined (by incorporation of BrdU and flow cytometry, respectively). Cytokine concentration in FLS culture supernatants was determined by ELISA. The Wilcoxon signed-rank test was used for statistical analysis.

Results HMW adiponectin enhanced IL-6, IL-8, VEGF ($p < 0.001$) and TGF β ($p < 0.01$) production. LMW adiponectin increased secretion of IL-6 and VEGF ($p < 0.001$), but its influence was much weaker than HMW's. TNF was the most potent in stimulating IL-6 and IL-8 production by ASCs ($p < 0.001$). The influence of adipocytokine treated-ASC-conditioned media on FLS was assessed by comparison with two controls: untreated ASC-conditioned medium and control FLS medium. ASC-conditioned medium treated with HMW caused down-regulation of IL-6 secretion by FLS comparing to both controls ($p < 0.05$). TNF-treated ASC-conditioned medium induced significant increase in MMP-3 production by FLS comparing to both controls ($p < 0.05$). Proliferation of FLS was up-regulated after untreated ASC-conditioned medium ($p < 0.01$) and this was partly reversed by HMW and TNF-treated ASC-conditioned media ($p < 0.05$). HMW-treated ASC-conditioned medium increased percentage of FLS in late apoptosis ($p < 0.05$).

Conclusions HMW and TNF seem to be the most potent in altering RA-ASCs properties. Leptin had no effect and LMW exerted only slight effect. HMW was able to up-regulate factors thought to mediate ASCs immunosuppression (TGF β , IL-6). In addition, its effect on FLS shows that this adipokine may exert positive effect on immunosuppressive ASCs function. However, because HMW up-regulated also proangiogenic cytokines (IL-8, VEGF), it is difficult to determine its real impact on RA-ASCs. TNF role in modulating RA-ASCs also need to be elucidated.

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5. B cells and autoimmunity

A5.1 ABNORMAL CALCIUM INFLUX IN T AND B LYMPHOCYTES FROM SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS IS RELATED TO STIM-1 OVER-EXPRESSION

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Background and Objectives Recently described, the molecule STIM1 (stromal interaction molecule 1) acts as a key mediator of calcium influx by controlling cell proliferation after antigen stimulation, Erk phosphorylation, cytokine production and apoptosis. Although STIM1 mutations have been associated with severe immunodeficiency, no study has focused on the STIM1 molecule in autoimmune diseases.

Materials and Methods T and B lymphocytes, purified by negative selection from peripheral blood of patients with systemic lupus erythematosus (SLE, $n = 11$), rheumatoid arthritis (RA, $n = 7$), primary Sjögren's syndrome (pSS, $n = 11$) and healthy controls (HC, $n = 12$) were tested by flow cytometry and Western blotting to determine the expression of STIM1. Video microscopy using specific probes has been used to assess intracellular calcium levels.

Results T cells from peripheral blood of HC express more STIM1 molecules (mean fluorescence intensity (MFI) 3.42 ± 0.13) than B cells (MFI 2.18 ± 0.20 , $P < 0.01$). In B lymphocyte subpopulations, the expression of STIM1 is 2 times higher in CD24^{high}CD38^{high} transitional B cells (MFI 4.83 ± 0.63) compared with CD24^{low}CD38^{low} mature B cells (MFI 2.47 ± 0.15 , $P < 0.01$) and CD24^{high}CD38^{low} memory B cells (MFI 3.64 ± 0.42 , $P < 0.05$). The expression of STIM1 in T and B lymphocytes from patients with RA and pSS was similar to HC.

An highest calcium influx and a constitutive Erk phosphorylation characterise T and B cells from SLE patients when compared with HC and disease controls. As suspected, STIM1 is over-expressed in SLE, when compared with HC and this expression is similar between T cells (MFI 8.70 ± 0.87) and B cells (MFI 9.00 ± 1.08). Within B cell subsets, STIM1 expression is 3.4 fold highest in transitional SLE B cells (MFI: 16.25 ± 2.18 , $P < 0.001$) than in transitional HC B cells and 2.3 fold highest than in mature SLE B cells (MFI: 7.1 ± 1.53) and memory SLE B cells (MFI: 9.51 ± 2.01). Western blotting results confirm the highest expression of STIM1 in SLE. Transient transfection of STIM1-targeting siRNAs was shown to restore the calcium influx and decrease Erk phosphorylation. Of particular note, the associations of CpG/anti-IgM Ab and CpG/anti-CD40 Ab are effective to induce STIM1 expression. Finally, STIM1 level was not correlated with the SLE disease activity index (SLEDAI) and autoantibodies (ANA, anti-dsDNA, anti-SSA/Ro).

Conclusions These results suggest that the differential expression of STIM1 may be an important factor in the process of lymphocyte self-reactivity in SLE, which opens new pathophysiological and therapeutic perspectives.

A5.2 ACCUMULATION OF CIRCULATING AUTOACTIVE NAÏVE B CELLS REVEALS DEFECTS OF EARLY B CELL TOLERANCE CHECKPOINTS IN PATIENTS WITH SJÖGREN'S SYNDROME

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Background and Objectives Sjögren's syndrome (SS) is an autoimmune disease characterised by high affinity circulating autoantibodies and peripheral B cell disturbances with predominance of naïve and reduction of memory B cells. The stage at which errors in B cell tolerance checkpoints accumulate in SS is unknown. Here we determined the frequency of self- and poly-reactive B cells in the circulating naïve compartment of SS patients.

Materials and Methods Single CD27-IgD+ B cells were sorted by FACS from peripheral blood of SS patients and healthy donors (HD). RNA was used to amplify Ig VH and VL genes and PCR products were cloned and expressed as recombinant monoclonal antibodies displaying identical specificity of the original B cells. Recombinant antibodies were tested towards different antigens to determine the frequency of autoreactive and polyreactive clones.

Results 66 recombinant antibodies were generated from naïve B cells of 4 SS patients and compared to 45 clones from 2 HD. Analysis of the VH and VL gene usage showed no significant differences between SS and HD. Conversely, we observed accumulation of circulating autoreactive naïve B cells in SS as demonstrated by increased reactivity towards Hep2 cells (43.1% SS versus 25% HD) and ENA (19.6% SS clones versus none). Among ENA+ clones, 6 displayed reactivity towards Ro/SSA and/or La/SSB.