The colocalisation between CD3ζ and SLAP were measured by laser confocal microscopy. CD3ζ mRNA was measured by quantitative real-time RT-PCR, IL-2 level was measured by ELISA.

Results in vitro TNF treatment of human T cells selectively, dose dependently and reversibly downregulates CD3 ζ-chain expression and inhibits activation-induced IL-2 expression ($p < 0.01$). Inhibition of the proteasome prevented the effect of TNF on CD3 ζ-chain expression. The colocalization of SLAP with CD3 ζ-chain and the SLAP expression were enhanced by TNF treatment ($p < 0.01$ and $p < 0.05$, respectively). SLAP silencing with small interfering RNA inhibited the TNF-induced ζ-chain downregulation. SLAP levels of the CD4 T cells, isolated from patients with rheumatoid arthritis were higher than that of the healthy donors' ($p < 0.05$). In addition, in vitro TNF treatment did not alter the SLAP expression of the CD4 lymphocytes of anti-TNF therapy-treated RA patients.

Conclusions our present data suggest that TNF regulates T cell activation during inflammatory processes, by altering CD3 ζ-chain expression via a SLAP-dependent mechanism. Thereafter SLAP-dependent regulation of CD3 ζ-chain may have an important role in the fine control of TCR signalling during chronic inflammation. SLAP may have a role in the pathomechanism of RA.

A3.21 TNFα INFLUENCES *RasGRP1* AND *RasGRP3* EXPRESSION LEVELS IN PBMC, B AND T CELLS

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Background Rheumatoid arthritis (RA) is the most common inflammatory arthritis. B and T lymphocytes play a central role in the pathophysiology of RA. *RasGRP* is a member of the CDC25 family of Ras guanyl nucleotide exchange factors. *RasGRP1* is expressed in T and B cells whereas *RasGRP3* is only expressed in B cells. In previous studies, we have shown that *RasGRP3* expression level significantly decreased in Peripheral blood mononuclear cells (PBMC) from RA patients responders to adalimumab after 3 months, leading to the question of TNFα involvement in pathways including RasGRP1 and RasGRP3.

Objectives To study TNFα effects on *RasGRP1* and *RasGRP3* expression levels in vitro.

Methods We measured by qRT-PCR, *RasGRP1* and *RasGRP3* expression levels, i) in PBMC from 3 healthy controls (HC), ii) in negative selected B and T cells from PBMC isolated from 3 buffy coat. In each condition, cells were cultured with or without BCR or TCR stimulation for 4 days and TNFα was added for 24 or 48 hours. Immunofluorescence staining was performed to cheque the cell purity and B and T cells stimulation by flow cytometry. To test the functionals effects of *RasGRP1* and *RasGRP3* overexpression in T and B cells respectively, IL-2 production was measured by ELISA in T-cells, and *Elk-1* expression level was measured by qRT-PCR in B cells before and after TNFα stimulation. In addition, TNFα effects on cell proliferation were evaluated by [^{3}H] thymidine incorporation by the B and T cells.

Results In B cells, TNFα induced an increase of *RasGRP1* ($p < 0.001$) and *RasGRP3* ($p < 0.001$) expression levels in absence of BCR stimulation. In the same way, in T cells, TNFα induced an increase of *RasGRP1* ($p < 0.001$) and *RasGRP3* ($p < 0.001$) expression levels in absence of TCR stimulation. Furthermore, TNFα induced a significantly increase of IL-2 production ($p < 0.05$) in unstimulated T cells and of *Elk-1* expression level ($p < 0.01$) in unstimulated B cells. However, TNFα have no effects on B and T cells proliferation.

Conclusions This study suggests the *RasGRP1* and *RasGRP3* regulation by TNFα, independently of B and T cells stimulation. The increasing of *RasGRP3* and *RasGRP1* in B and T cells specifically *via* TNFα binding on its receptors could promote the activation and proliferation of B and T cells by an independent antigen pathway. This second pathway could explain the maintenance of B and T cells activation.

A3.22 UPREGULATED MICRORNA-182 EXPRESSION IS ASSOCIATED WITH ENHANCED CONVENTIONAL CD4$^+$ T CELL PROLIFERATION IN SLE

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Background Recent reports have shown dysregulated microRNAs (miRNAs) in murine models of lupus, among them increased expression of miRNA-182, which has been demonstrated to target the transcription factor *FOXO1* in activated murine CD4$^+$ T cells. The loss of *FOXO1* activity in T cells is associated with spontaneous T cell activation, clonal expansion and autoantibody production, all of which are present in systemic lupus erythematosus (SLE).

Methods Expression levels of microRNA-182 (miR-182) and *FOXO1* were analysed with RT-PCR in freshly isolated and magnetic purified peripheral blood CD4$^+$ T cells from 9 patients with SLE and age/sex-matched healthy controls (HC). Multicolor flow cytometry was performed to analyse CD4$^+$ T cell expression for CCR7, CD45RA, Ki-67, Foxp3, the interleukin-7 receptor-α (CD127) and phosphorylated STAT-5a (pSTAT5). Analysis of serum IL-7 levels was performed with ELISA in 27 SLE patients and HC (R&D systems). The Wilcoxon signed-rank test was used for statistical analysis.

Results MiRNA-182 was significantly upregulated in CD4$^+$ T cells from SLE patients compared to HC (median relative expression 8.89 × 10E-7 versus 3.96 × 10E-7, $p = 0.008$) while *FOXO1* mRNA levels were decreased, yet without reaching statistical significance. Analysis of Ki-67 expression revealed an increased percentage of proliferating CD4$^+$ T cells in SLE (5.23% versus 2.21%, $p = 0.006$), which was more prominent in Foxp3$^-$ conventional T cells (Tcons) than in Foxp3$^+$ regulatory T cells (Tregs). Overall, CD4$^+$ T cellular proliferation in SLE was associated with increased frequencies of CD45RA$^-$ CCR7$^-$ effector memory T cells and enhanced basal pSTAT5 levels (median MFI 503.5 versus 399.0, $p = 0.010$), suggesting a recent stimulation with common gamma chain(γc)-signalling cytokines. In this regard, Tcons from SLE samples displayed decreased expression levels for the *FOXO1* target gene CD127 (MFI 2021 versus 2553, $p = 0.049$) and serum IL-7 levels were significantly higher in SLE when compared to HC (17.0 pg/ml versus 10.2 pg/ml, $p = 0.001$).

Conclusions MiR-182 expression has been shown to be directly dependent on STAT5 activation and to promote the clonal expansion of murine activated CD4$^+$ T cells. Our data suggest that enhanced IL-7R/STAT5 signalling presumably mediates the induction of miR-182 expression, which in turn promotes the proliferation of Tcons in SLE. The relative contribution of IL-7R/miR-182/FOXO1 axis on the enhanced proliferative capacity of SLE Tcons remains elusive and merit further investigation. Collectively, our data provide new insights in the pathophysiology of T cell hyperactivity in SLE and identifies miR-182 as a candidate target for future therapeutic approaches.