A3.23 VITAMIN D ANTAGONISES THE SUPPRESSIVE EFFECT OF INFLAMMATORY CYTOKINES UPON CTLA-4 EXPRESSION AND REGULATORY FUNCTION

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Background and Objectives The suppressive protein, cytotoxic T lymphocyte antigen–4 (CTLA-4), is constitutively expressed by TRegs and induced in effector T cells upon activation. Its crucial role in adaptive immunity is apparent from the fatal autoimmune pathology of CTLA-4 knockout mice and the association of CTLA-4 genetic variants with autoimmunity. We have recently shown that CTLA-4 functions by depleting antigen-presenting cells of their co-stimulatory ligands, CD80 and CD86 (Qureshi *et al*, Science 2011). However, little is known about the factors that regulate CTLA-4 expression and function. Since low vitamin D status and elevated Th17 frequencies are evident in many autoimmune conditions, we have investigated the effect of vitamin D and Th17 polarising cytokines upon CTLA-4 expression and function.

Methods Peripheral blood $CD4^+CD25^-$ T cells were stimulated under Th17 polarising conditions (TGFbeta, IL-1beta, IL-6 and IL-23) with or without active vitamin D ($1.25(OH)_2D_3$). FoxP3 and CTLA-4 were measured by flow cytometry and the vitamin D receptor (VDR) by qPCR. To assess CTLA-4 function, T cells were cultured with anti-CD3 and cells expressing GFP-tagged CD86. CD86-GFP acquisition by T cells with or without CTLA-4 blockade was then monitored by flow cytometry. For suppression assays, separately labelled activated T cells and $CD4^+CD25^-$ responder T cells were co-cultured with dendritic cells in the presence of anti-CD3 and T cell proliferation assessed at five days by flow cytometry.

Results Vitamin D increased CTLA-4 expression and the frequency of FoxP3+CTLA-4+ T cells. By contrast, Th17 polarising cytokines suppressed CTLA-4. Interestingly, when supplied together, Th17 polarising cytokines synergised with vitamin D resulting in significantly higher CTLA-4 expression than with vitamin D alone. This synergy corresponded with increased VDR expression under Th17 conditions. Using a novel assay to test CTLA-4 function, we further confirmed that these changes in CTLA-4 expression correlated with ligand removal. Moreover, in dendritic cell driven stimulations vitamin D-treated T cell blasts showed enhanced CTLA-4-mediated suppression.

Conclusions Vitamin D overrides the inhibitory effect of pro-inflammatory Th17 polarising cytokines upon CTLA-4 expression and function. Given the importance of CTLA-4-mediated suppression in the control of autoimmune diseases, including RA, these data highlight the importance of vitamin D in immune regulation and its potential as a therapeutic agent.

A3.24 ALTERATIONS IN γδ T CELLS HOMEOSTASIS IN SYSTEMIC SCLEROSIS

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Background Systemic Sclerosis (SSc) is an autoimmune disease characterised by inflammation and extracellular matrix deposition. There are growing evidences of immune cells alterations, mainly in T cells subsets, including γδ T cells.

Objectives Evaluate the frequency of γδ T cells, their distribution among naïve, memory and effector functional compartments, the cytotoxic activity and γδ repertoire in SSc patients.

Materials and Methods The study enrolled 43 SSc patients, 30 of limited subtype (lSSc) and 13 of diffuse subtype (dSSc). Additionally 14% of the patients had less than 1 year since diagnosis, 49% ranging between 1 and 10 years and 37% with more than 10 years since diagnosis. The healthy control group (HG) included 20 age and gender matched individuals. The phenotypic and functional activity of γδ T cells was assessed by flow cytometry.

Results It was observed a lower frequency of γδ T cells in SSc patients compared to HG. Regarding the cytotoxic activity, it was verified a higher frequency of γδ T cells expressing granzime B and perforin in SSc group, particularly in lSSc, with a higher γδ T cells cytotoxic activity in patients with more than 10 years since the disease onset. Moreover, an increased frequency of γδ T cells with a naïve and effector phenotypes was observed in SSc group and these alterations were more evident in dSSc patients. Additionally, patients with pulmonary fibrosis demonstrated an increased frequency of effector memory and naïve γδ T cells closely associated to the decrease of central memory cells, when compared to patients without this clinical feature. Patients with more than 10 years since the disease onset, compared to those with less than 1 year since disease diagnosis, presented a higher frequency of effector cells associated to the decrease of central memory cells. Concerning γδ TCR repertoire assigned by Vδ2 and Vγ9 chains, it was observed in patients with SSc for more than 10 years, an increase of $V\delta2^-V\gamma9^-$ repertoire in naïve, central memory and effector γδ T cells when compared to patients with less than 1 year since diagnosis, which exhibit predominantly a $V\delta2^+V\gamma9^+$ and $V\delta2^+V\gamma9^-$ repertoire.

Conclusions Important alterations were observed in the frequency, cytotoxic activity, distribution among functional compartments and in γδ T cell receptor repertoire in peripheral blood γδ T cells that seem to be related to the time of disease after diagnosis or to clinical findings.

A3.25 DELETION OF RBP-J IN A MURINE MODEL OF INFLAMMATORY ARTHRITIS REVEALS DIFFERENTIAL PRO-INFLAMMATORY CYTOKINE AND FOXP3 GENE EXPRESSION

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Background/Purpose The DNA-binding protein RBP-J serves as the central transcriptional regulator of the Notch signalling pathway. Prior work done using a knockdown approach of RBP-J in human macrophages and conditional deletion of RBP-J in mouse macrophages has demonstrated diminished LPS-induced expression of TNFα, IL-6 and IL-12p40, with IL-1 induction preserved. Elsewhere, it has been observed that host regulatory T cells in RBP-J deficient mice have an attenuated ability to suppress effector T cell responses in vitro, with augmented proliferation and function of effector T cells noted in vivo, raising the possibility that dysregulation in the frequency or function of regulatory T cells may contribute to RBP-J's selective modulation of pro-inflammatory mediators. Here, we evaluated the in vivo effects of RBP-J's conditional deletion in the myeloid cell compartment on pro-inflammatory cytokine expression, as well as lymphoid tissue immunocyte composition, using a K/BxN serum transfer model of inflammatory arthritis.

Methods $RBP\text{-}J^{flox/flox}$ LysM-Cre knock-out (KO) mice with littermate $RBP\text{-}J^{+/+}$ LysM-Cre controls (n = 5 for each group) were used. After treatment with K/BxN serum, the clinical course of arthritis was followed by measuring total joint thickness up to 14 days, at which point the mice were sacrificed. Total joint RNA from each

mouse was obtained for gene expression analyses by qPCR. Splenic tissue was harvested from each mouse for gene expression analyses by qPCR, as well as pooled collectively for each group for immunophenotyping through flow cytometry. The latter was also done for superficial inguinal and draining popliteal lymph node (LN) tissue. Statistical analysis was done using the unpaired student's t-test with $p < 0.05$ considered significant.

Results Preliminary findings showed no significant difference in clinical phenotype of K/BxN serum-induced arthritis between KO and control mice. Gene expression profiling of whole joint tissue showed decreases in TNFα, IL-6, IFN-γ expression, as well as selective Notch target gene expression, while maintaining comparable IL-1, CXCL10, and IL-12p40 levels. Surprisingly, expression levels of FoxP3 in KO mice versus controls were significantly decreased in both joint and splenic tissue ($p = 0.0244$ and $p = 0.0286$, respectively). Immunophenotyping of splenic and LN tissue showed increased proportions of CD4+ T cells in KO mice versus controls, but a markedly lower proportion of CD4+CD25+FoxP3+ cells. Lower proportions of F4/80+ and Ly6G+ cell populations in splenic and draining LN tissue of KO mice versus controls, but higher populations of Ly6C+ cells, were also observed.

Conclusions Deletion of RBP-J in the myeloid compartment does not lead to phenotypic differences in K/BxN serum-induced inflammatory arthritis, though selective modulation of pro-inflammatory cytokine gene expression in vivo does occur. Decreased gene expression of FoxP3 and fewer CD4+CD25+FoxP3+ cells in RBP-J deleted mice may contribute to this selective modulation. The functional significance of these findings, coupled with differences in myeloid cell composition and trafficking observed, remain undetermined and will be further studied.

A3.26 IDENTIFYING T-FOLLICULAR-HELPER-LIKE CELL INVOLVEMENT IN THE ORGANIZATION OF TUBULOINTERSTITIAL INFLAMMATION IN HUMAN LUPUS NEPHRITIS AND RENAL ALLOGRAFT REJECTION

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Background Tubulointerstitial inflammation (TII) is a usual feature of lupus nephritis (LuN) and the infiltrate is organised into well-circumscribed T:B-cell aggregates or germinal centre-like structures.

Materials and Methods We analysed 42 LuN biopsies using confocal laser scanning microscopy (CLSM) for the qualitative presence of T_{FH}-like $CD4^+ICOS^+$ cells (T_{IH})(n = 19 for ICOS-positive cases) and compared this with T-cell-mediated (TCM, n = 8) and mixed renal allograft (mixed RT, n = 7) rejection cases. Cellular make-up and relationships within TII were examined using CLSM to identify and compare the locations of T_{IH} cells and $CD20^+$ B cells by means of a computerised cell-distance algorithm.

Results The prevalence of T_{IH} cells was found to be 45.2% in LuN, 64.2% in TCM, and 50% in mixed RT cases. Presence of these cells in LuN was statistically correlated with a worse grade of TII as scored by a blinded pathologist (2.05 versus 1.48, $p = 0.04$). ICOS-positive biopsies were associated with a higher mean serum creatinine in adult patients at time of biopsy (2.3 versus 1.1 mg/dL, $p = 0.03$) as well as GFR as measured by the MDRD equation and adjusted for patient sex (44.8 versus 74.1 mL/min/1.73 m^2, $p = 0.04$) with no statistical differences in age, sex, ISN/RPS LuN class, or NIH activity or chronicity indices.

Cellular distance mapping revealed that T_{IH} cells were spatially related to $CD20^+$ B cells across LuN (42.9% of cells within 0.27 microns, n = 10 biopsies), control tonsil tissue (65.3%, n = 2 biopsies), and mixed RT cases (70.3%, n = 7 biopsies) as compared to other T cells (less than 20% for all groups, respectively). These associations remained unchanged after correction for total cellular density and T:B cell ratios. Low-density TCM cases showed a comparatively low rate of T_{IH}:B cell association (15.0%, n = 8 biopsies) and these results were statistically significant ($p < 0.0001$ versus mixed RT and tonsil cases, $p = 0.002$ versus LuN). Comparing the above results against a theoretical model of random T_{IH}:B-cell distribution revealed that the likelihood of our observations in LuN being due to chance was approximately 8.12×10^{-41}.

Conclusions Our data reveal that T_{IH} cells are present in similar rates across cases of renal allograft rejection as well as LuN. Their presence is associated with a higher degree of TII as well as worse renal function at time of biopsy in LuN. T_{IH} cells are more likely to form proximal conjugates with naïve and activated B cells in tissues of diseases associated with aberrant autoantibody production (SLE, mixed RT) but not in processes where autoantibodies are absent (TCM).

4. Metabolism, hormones and autoimmunity

A4.1 1.25(OH)$_2$D$_3$ MODULATES GENE EXPRESSION INVOLVED IN PHENOTYPE STABILITY AND MIGRATION OF TH17 CELLS FROM PATIENTS WITH RHEUMATOID ARTHRITIS

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Background and Objectives Vitamin D has suppressive effects on autoimmune diseases, such as rheumatoid arthritis (RA). Within these diseases, T-helper-17 (Th17) cells have been implicated to play a crucial role in the development and progression of chronic inflammation. Recently, we have found that the active vitamin D compound, 1.25(OH)$_2$D$_3$, has direct suppressive effects on both human and mouse Th17 cytokine expression and activity. Using gene-expression profiling, we aim to identify molecular targets of 1.25(OH)$_2$D$_3$ signalling underlying this suppressive action of 1.25(OH)$_2$D$_3$ in Th17 cells.

Methods Primary Th17 cells were sorted from peripheral blood of treatment naïve patients with early RA and cultured with or without 1.25(OH)$_2$D$_3$. From these cultures gene-expression profiles were generated. Expression of genes of interest was confirmed by Q-PCR and/or specific ELISA.

Results In the presence of 1.25(OH)$_2$D$_3$, protein expression of Th17 associated cytokines IL-17A and IL-22 was inhibited, while in contrast the anti-inflammatory cytokine IL-10 was induced. These findings were supported by the gene-expression profiles from these cultures. Furthermore, 1.25(OH)$_2$D$_3$ inhibited transcription of the cytokine receptors IL-23R and IL-7R, which are involved in Th17 survival and proliferation. Chemokines CCL20 and CXCL10 were down-regulated and chemokine receptors CCR2, CXCR6, CXCR3 and CCR10 were up-regulated. Importantly, RORγt, which is critically involved in Th17 differentiation and function and the cell-size regulator and oncogene c-Myc were down-regulated by 1.25(OH)$_2$D$_3$.

Conclusions: From these findings, we concluded that 1.25(OH)$_2$D$_3$ modulates the expression of genes involved in cytokine production, proliferation, and migration of Th17 cells. These data indicate that 1.25(OH)$_2$D$_3$ not only suppresses Th17 cell activity but also regulates Th17 phenotype stability and migration of these cells to sites of tissue inflammation in RA.