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

Background and Objectives The suppressive protein, cytotoxic T lymphocyte antigen-4 (CTLA-4), is constituently expressed by TReg cells 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 (Oureshi 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)2D3). FoxP3 and 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.

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 polarising conditions. Using a novel assay to test CTLA-4 function, we further confirmed that these changes in CTLA-4 expression were associated 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.

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

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 immuneocyte composition, using a K/BxN serum transfer model of inflammatory arthritis.

Methods RBP-Jflox/flox LysM-Cre knock-out (KO) mice with littermate RBP-Jflox/flox 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 group and controls was extracted and subjected to quantitative real-time PCR analysis. Results Statistical analysis was performed using a one-way ANOVA with Bonferroni correction.

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