A105 25-HYDROXYVITAMIN D_3 CONVERSION BY DENDRITIC CELLS AND T CELLS DRIVES 1,25-DIHYDROXYVITAMIN D_3 MEDIATED ANTI-INFLAMMATORY $CD4^+$ T CELL RESPONSES

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Background Low vitamin D status is associated with an increased risk of autoimmune diseases, including rheumatoid arthritis. Thus, understanding how vitamin D modifies immune reactions holds therapeutic potential. The authors have shown that $1,25(OH)_2D_3$, acts directly upon human CD4 T cells, suppressing inflammatory cytokines (interleukin (IL)-17, IL-21, IFNγ and IL-22) while enhancing regulatory-markers (CTLA-4, CD25, FoxP3 and IL-10). However, the short half life of $1,25(OH)_2D_3$ and its low serum level imply that local conversion of $25(OH)D_3$, the major circulating form of vitamin D, by 1α-hydroxylase (CYP27B1), is necessary for immune regulation in vivo. Thus, the authors have studied the effect of $25(OH)D_3$ upon T cell responses. In view of the critical role of CTLA-4 in immune-regulation and its strong sensitivity to $1,25(OH)_2D_3$, the authors have also investigated CTLA-4 function in vitamin D-modified immune responses.

Methods $CD4^+CD25^-$ T cells were purified from human peripheral blood (PB) and stimulated with LPS-matured allogenic dendritic cells (DCs) plus anti-CD3 or with anti-CD3/CD28 beads in the presence and absence of $25(OH)D_3$. FoxP3, CTLA-4 and cytokines were measured by flow cytometry and CYP27B1 by qPCR. In function assays, CTLA-4 was blocked with anti-CTLA-4 and T cell division monitored. DC expression of CD80 and CD86 was assessed by FACS. PB and synovial fluid (SF) mononuclear cells, from patients with synovitis, were stimulated with antiCD3 in the presence and absence of $1,25(OH)_2D_3$ and cytokines examined by FACS.

Results Stimulation of T cells by DCs in the presence of $25(OH)D_3$ led to strong suppression of IL-17 and IFNγ but upregulation of CTLA-4 and $CTLA\text{-}4^+FoxP3^+$ frequencies. By contrast, in the absence of DCs, $25(OH)D_3$ caused modest CTLA-4 induction and IFNγ suppression ($p<0.05$ for all). These differences corresponded with CYP27B1 levels, as maturation induced much higher CYP27B1 in DCs than arose in T cells ($p<0.05$). CTLA-4 blockade overcame $1,25(OH)_2D_3$-mediated suppression of T cell division in DC stimulations and prevented downregulation of co-stimulatory CD80 and CD86 on DCs. PB T cells from synovitis patients responded normally to $1,25(OH)_2D_3$ with suppressed IL-17 and IFNγ ($p<0.0001$). $1,25(OH)_2D_3$ also reduced IL-17 ($p<0.05$) but not IFNγ in SF T cells.

Conclusions The authors have shown that DCs can efficiently convert $25(OH)D_3$ to drive $1,25(OH)_2D_3$-modified T cell responses and that upregulation of CTLA-4 is mechanistically important in immune suppression by vitamin D. Thus, $25(OH)D_3$ supplement could be useful in the treatment of conditions such as RA. This is supported by the authors' finding that T cells from synovitis patients can respond to $1,25(OH)_2D_3$.