EXTENDED REPORT

TNF blockade requires $1,25(OH)_2D_3$ to control human Th17-mediated synovial inflammation

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ABSTRACT

Objectives T helper 17 (Th17) cells from patients with early rheumatoid arthritis (RA) induce a proinflammatory feedback loop upon RA synovial fibroblast (RASF) interaction, including autocrine interleukin (IL)-17A production. A major challenge in medicine is how to control the pathogenic Th17 cell activity in human inflammatory autoimmune diseases. The objective of this study was to examine whether tumour necrosis factor (TNF) blockade and/or 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) controls Th17-mediated synovial inflammation.

Methods Peripheral CD4+CD45RO+CCR6+ Th17 cells of patients with early RA, Th17–RASF cocultures and synovial biopsy specimens were cultured with or without $1,25(OH)_2D_3$ and/or TNFα blockade. Intracellular cytokine expression was detected by flow cytometry. Cytokine and matrix metalloprotease (MMP) production was determined by ELISA.

Results The authors show that the $1,25(OH)_2D_3$, but not TNFα blockade, significantly suppressed autocrine IL-17A production in Th17–RASF and synovial biopsy cultures. Combining $1,25(OH)_2D_3$ and TNFα blockade had a significant additive effect compared with single treatment in controlling synovial inflammation, indicated by a further reduction in IL-6, IL-8, MMP-1 and MMP-3 in Th17–RASF cocultures and IL-6 and IL-8 expression in cultures of RA synovial tissue.

Conclusions These data show that TNF blockade does not suppress IL-17A and IL-22, which can be overcome by $1,25(OH)_2D_3$. The combination of neutralising TNF activity and $1,25(OH)_2D_3$ controls human Th17 activity and additively inhibits synovial inflammation. This indicates more valuable therapeutic potential of activation of Vitamin D receptor signalling over current TNF neutralisation strategies in patients with RA and potentially other Th17-mediated inflammatory diseases.

The cause of rheumatoid arthritis (RA) is largely unknown. However, substantial evidence has emerged supporting the role of T cells and their cytokines in RA initiation and progression.[1–3] In particular, interleukin (IL)-17A-producing T helper 17 (Th17) cells are attractive targets for RA treatment.[4 5] Th17 cells are further characterised by IL-17F and IL-22 production and C-C chemokine receptor6 cell-surface expression.[6–8] The pathogenic role of IL-17A in murine arthritis has been identified.[9] Moreover, mice deficient in factors underlying Th17 differentiation or function are protected against induction and/or progression of arthritis.[10 11] In the RA-inflamed joint synovium, IL-17A is expressed and IL-17A-producing T cells have been identified.[12–16] In addition, we have found increased CCR6+ Th17 cell percentages in peripheral blood of treatment-naive patients with early RA.[17] The pathogenic potential of IL-17-producing cells including Th17 cells is further indicated by a decline in disease activity of patients with RA in a clinical anti-IL-17 trial.[18]

We recently showed that Th17 cells were potent activators of RA synovial fibroblast (RASF).[17] This Th17–RASF interaction revealed a potential Th17 pathogenic activity as shown by: (1) increased production of IL-6 and IL-8 and matrix metalloprotease (MMP)-1 and MMP-3, mediators of cartilage degradation; (2) induction of autocrine IL-17A production, indicating a Th17-induced proinflammatory loop. This loop may be an important pathway in the progression of early inflammatory arthritis to chronic persistent arthritis.[17]

A major challenge in medicine is how to control pathogenic Th17 cell activity in human autoimmune-mediated diseases. Interestingly, the active vitamin D metabolite, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), has been shown to affect murine Th17 cytokine expression and function.[19 20] The suppressive effects of $1,25(OH)_2D_3$ have been mainly linked to the modulation of functional activities of antigen-presenting cells (APCs).[21 22] Although T cell polarisation can indirectly be affected by $1,25(OH)_2D_3$-induced altered APC cytokine expression,[23 24] it is becoming clear that T cells, including Th17 cells, are direct $1,25(OH)_2D_3$ targets.[25–27] In human T cells, $1,25(OH)_2D_3$ suppresses IL-17A and interferon γ (IFNγ) production and stimulates IL-4 and IL-10 production.[28–30] Moreover, $1,25(OH)_2D_3$ directly reduced the production of the Th17 cytokines, IL-17A, IL-17F and IL-22, by memory T cells of patients with early RA.[27] The proinflammatory cytokine, tumour necrosis factor α (TNFα), is a commonly used target for RA treatment.[2] However, $1,25(OH)_2D_3$ had no effect on TNFα production by stimulated peripheral blood mononuclear cells, and only limited inhibitory effects on TNFα production by memory T cells.[27]

Since $1,25(OH)_2D_3$ has been shown to inhibit IL-17A production by T cells, we hypothesised that

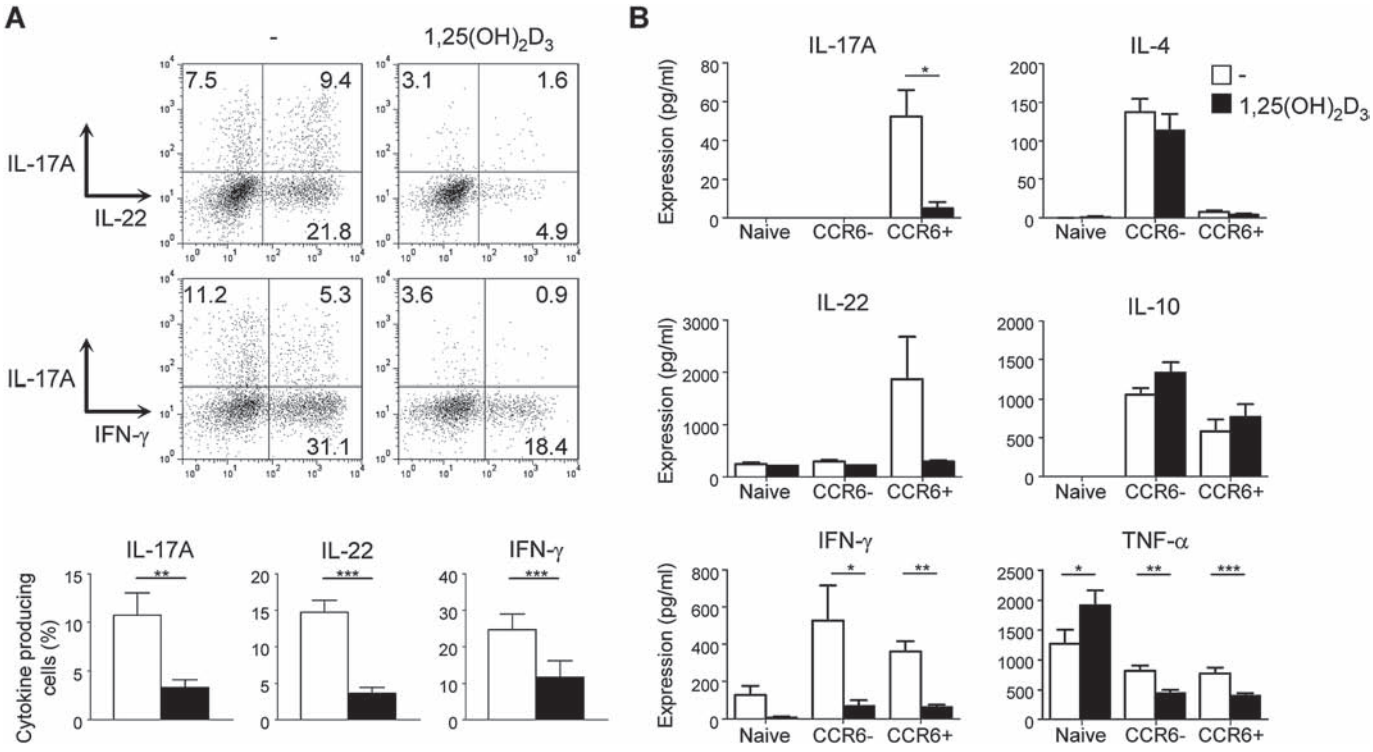

Figure 1 Effects of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) on T helper 17 (Th17)-, Th1- and Th2-associated cytokine expression by peripheral T cells of patients with early rheumatoid arthritis (RA). Sorted memory CCR6+ Th17, memory CCR6− and naive T cells from peripheral blood were stimulated with αCD3/αCD28 and cultured for 3 days with or without $1,25(OH)_2D3$. (A) Flow cytometric analysis of intracellular interleukin (IL)-17A, IL-22 and interferon (IFN)γ expression by CCR6+ Th17 cells. Numbers in representative dot plots (upper panel) indicate the proportion of cytokine-expressing cells per quadrant. Mean and SEM (lower panel) are given for CCR6+ Th17 cells obtained from eight patients with early RA, cultured in the absence (white bars) or presence (black bars) of $1,25(OH)_2D_3$. (B) Expression of indicated cytokines in supernatant of memory CCR6+ Th17, memory CCR6− and naive T cells in the absence (white bars) or presence (black bars) of $1,25(OH)_2D_3$. Mean and SEM are given for five treatment-naïve patients with early RA. Results are representative of at least three independent experiments. *p<0.05; **p<0.01; ***p<0.001.

VDR pathway activation by $1,25(OH)_2D_3$ may directly regulate the pathogenic activity of Th17 cells from patients with RA. In addition, we hypothesised that $1,25(OH)_2D_3$ has an additional role to TNFα blockade in suppressing synovial inflammation through modulation of Th17 function. These hypotheses were tested using Th17–RASF cocultures from treatment-naïve patients with early RA or RA synovial biopsy cultures from patients with RA undergoing knee joint replacement.

Here we show direct suppressive effects of $1,25(OH)_2D_3$, but not of TNFα blockade, on IL-17A and IL-22 cytokine expression. Furthermore, the combination of neutralising TNF activity and $1,25(OH)_2D_3$ controls human Th17 activity and additively inhibits synovial inflammation. These data support the development of a clinical trial combining TNF blockade with VDR activation in RA and other Th17-mediated autoimmune diseases.

METHODS

Subjects

In this study, 16 treatment-naive patients with early RA (13 women and three men; mean±SD age 47.8±14.4 years) were studied. All patients fulfilled the American College of Rheumatology 1987 revised criteria for RA. Blood was obtained at the second visit after informed consent had been obtained. Clinical and laboratory data for the patients can be found in online supplementary table S1. Data from patients with established RA are available on request. This study was embedded in the Rotterdam Early Arthritis Cohort Study and approved by the medical ethics committee of the Erasmus MC Rotterdam.

Flow cytometry antibodies and cell sorting

Monoclonal antibody preparations, intracellular cytokine detection and flow cytometry have been described previously.[11] The following monoclonal antibodies were purchased from BD Biosciences (San Diego, California, USA): CD45RO, CCR6, CD4 and IFNγ. IL-22 and IL-17A monoclonal antibodies were from eBioscience (San Diego, California, USA). Samples were acquired on a FACScantoII flow cytometer (BD Biosciences) and analysed using FlowJo v7.6 research software (Tree Star Inc, Ashland, Oregon, USA). T cell populations were sorted from peripheral blood mononuclear cells using a FACSAria cell sorter (BD Biosciences).

Cell cultures

T cells (2.5×10^4) were cultured for 72 h in Iscove's modified Dulbecco's medium (BioWhittaker, Walkersville, MD, United States), supplemented with 10% fetal calf serum, 100 U/ml penicillin/streptomycin, 2 mM L-glutamine and 50 μM β-mercaptoethanol (Merck, Darmstadt, Germany). Cells were stimulated with soluble αCD3 and αCD28 (0.3 and 0.4 μg/ml, respectively; Sanquin, Amsterdam, The Netherlands) and cultured with or without 100 nM $1,25(OH)_2D_3$ (Leo Pharmaceuticals Products, Ballerup, Denmark).

RASF isolation and subsequent culture has been described.[17] RASFs (1.0×10^4) were cocultured with 2.5×10^4 allogeneic Th17 cells for 72 h with soluble αCD3/αCD28 and/or 100 nM $1,25(OH)_2D_3$ and/or 10 μg/ml etanercept (Wyeth Pharmaceuticals Inc, Collegeville, Pennsylvania, USA).

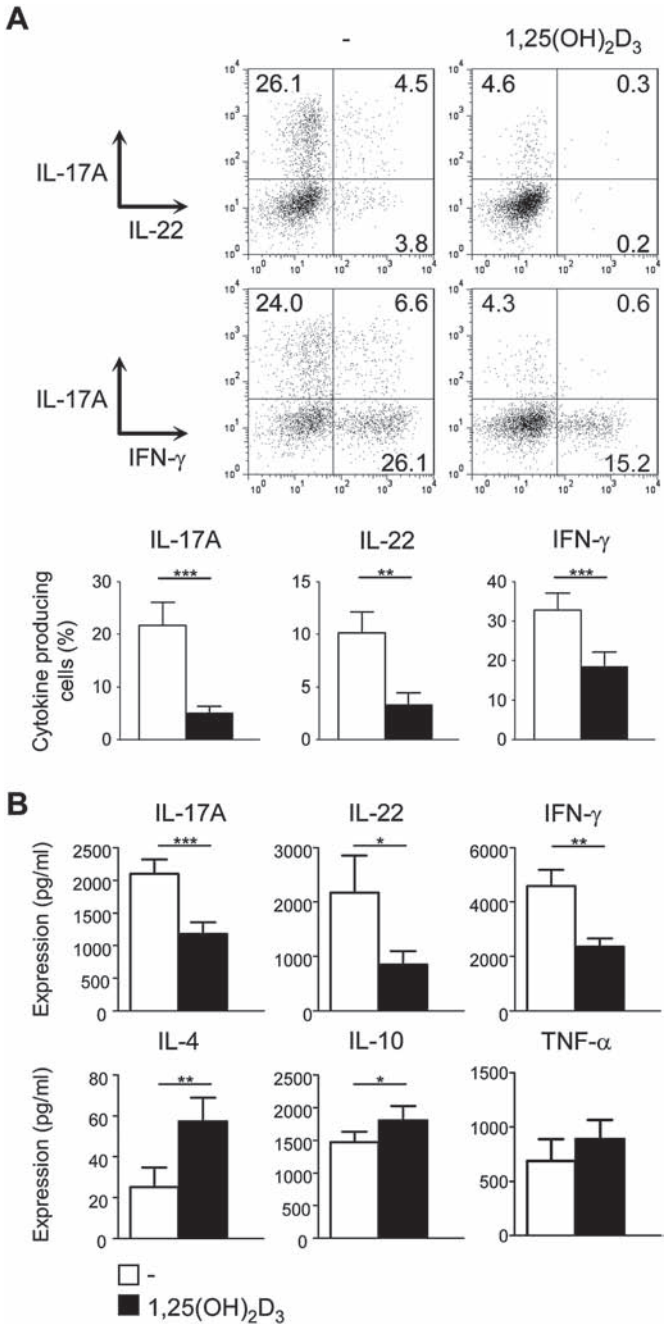

Figure 2 Effects of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) on the expression of T cell-associated cytokines in T helper 17 (Th17)–rheumatoid arthritis (RA) synovial fibroblast (RASF) cocultures. (A) Flow cytometric analysis for intracellular interleukin (IL)-17A, IL-22 and interferon (IFN)γ. Numbers in a representative dot plot (upper panel) represent the percentage of cytokine-producing cells in each quadrant. Mean and SEM (lower panel) are given for Th17–RASF cocultures stimulated with αCD3/αCD28 in the absence (white bars) or presence (black bars) of $1,25(OH)_2D_3$, whereby Th17 cells were obtained from five patients with early RA. (B) Expression of indicated cytokines in Th17–RASF cocultures in the absence (white bars) or presence (black bars) of $1,25(OH)_2D_3$. Mean and SEM are given for five treatment-naïve patients with early RA. Results are representative of at least three independent experiments. *p<0.05; **p<0.01; ***p<0.001.

Synovial biopsy specimens (~2 mm) were taken randomly from synovial tissue obtained from patients with established RA after knee joint replacement. Three biopsy samples per group were cultured for 72 h in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 100 U/ml

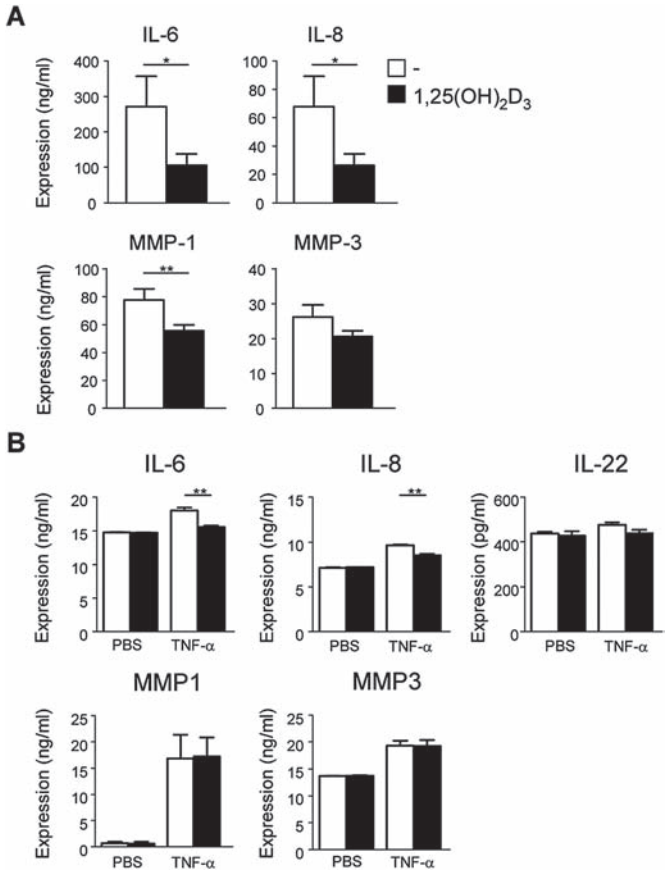

Figure 3 1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) inhibits interleukin (IL)-6, IL-8, matrix metalloprotease (MMP)-1 and MMP-3 expression in T helper 17 (Th17)–rheumatoid arthritis synovial fibroblast (RASF) cocultures. Detection of indicated cytokines by ELISA in supernatant of (A) Th17–RASF cocultures or (B) phosphate-buffered saline (PBS) or tumour necrosis factor (TNF)α (1 ng/ml)-stimulated RASF monocultures in the absence (white bars) or presence (black bars) of $1,25(OH)_2D_3$ and stimulated with αCD3/αCD28. (A) Mean and SEM are given for Th17–RASF cocultures, whereby Th17 cells were obtained from five patients with early rheumatoid arthritis. (B) Mean and SEM are given for three RASF cultures. Results are representative of at least three independent experiments. *p<0.05; **p<0.01.

penicillin/streptomycin, in the presence of αCD3/αCD28 and/or 100 nM $1,25(OH)_2D_3$ and/or 10 μg/ml etanercept.

Cytokine measurements

IL-4, IL-6, IL-8, IL-10 and IFNγ production was determined using ELISA (Invitrogen, Carlsbad, California, USA). IL-17A, IL-22, TNFα, MMP-1 and MMP-3 expression was measured using Duoset ELISA (R&D systems, Minneapolis, Minnesota, USA). ELISA was performed according to the manufacturer's instructions.

Statistical analysis

Differences between experimental groups were tested with a two-sided paired t test or stated otherwise, using Prism software V.5.04 (GraphPad Software Inc. La Jolla, California, USA). p Values <0.05 were considered significant.

RESULTS

$1,25(OH)_2D_3$ suppressed Th17-associated cytokine expression by primary memory CCR6+ Th17 cells from patients with early RA

Recently, we showed that $1,25(OH)_2D_3$ suppressed IL-17A and IL-22 expression by CD4+CD45RO+ (memory) T cells from

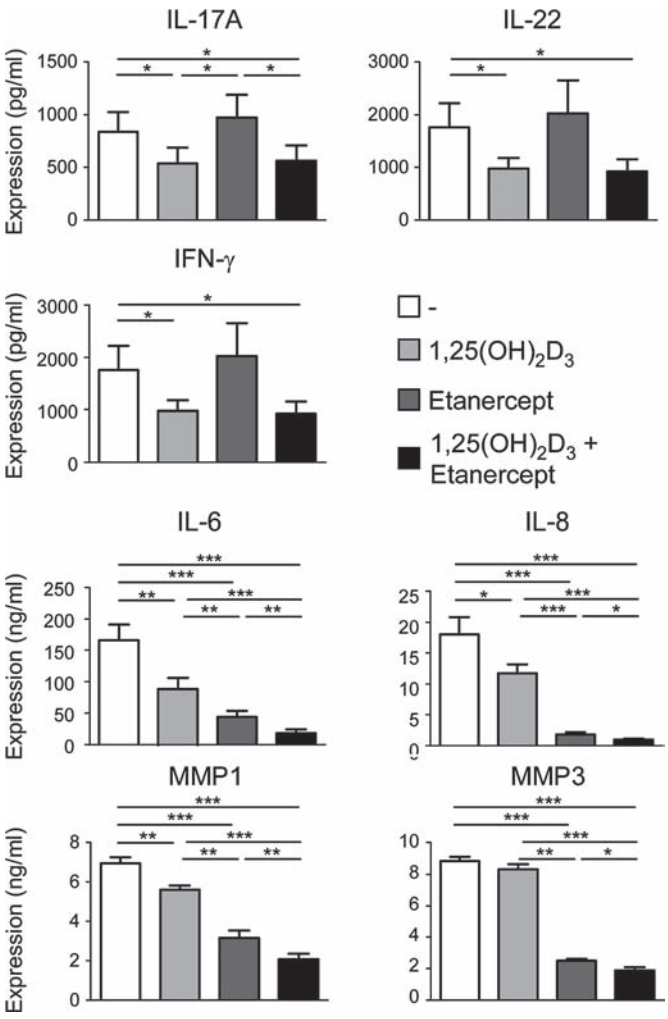

Figure 4 The combination of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and tumour necrosis factor α (TNFα) blockade has additional effects on interleukin (IL)-6, IL-8, matrix metalloprotease (MMP)-1 and MMP-3 expression compared with TNFα blockade alone in T helper 17 (Th17)–rheumatoid arthritis synovial fibroblast (RASF) cocultures. Expression of the indicated cytokines and MMPs was detected with ELISA in supernatant of Th17–RASF cocultures stimulated with αCD3/αCD28 in the presence or absence $1,25(OH)_2D_3$ and or TNFα blockade. Mean and SEM are given, whereby Th17 cells were obtained from five treatment-naïve patients with early rheumatoid arthritis. Results are representative of at least three independent experiments. *p<0.05; **p<0.01; ***p<0.001.

treatment-naive patients with early RA.[27] By using CCR6 expression, it is possible to sort primary IL-17A-producing memory T cells (CCR6+ Th17) from peripheral blood.[7 17] When these cells of patients with early RA were stimulated in the presence of $1,25(OH)_2D_3$, a significant reduction was found in the percentage of IL-17A- and IL-22-expressing T cells and in IL-17A and IL-22 protein levels in the supernatant (figure 1A,B). In both Th17 and CCR6 memory T cell cultures, $1,25(OH)_2D_3$ reduced the percentage of IFNγ-producing cells and IFNγ protein production (figure 1A,B).

$1,25(OH)_2D_3$ is known to induce IL-4 production in murine naive T cell cultures[31] and IL-10 production by T cells from patients with multiple sclerosis.[30] In CD4+CD45RO− (naive) T cell, memory CCR6− T cell and memory CCR6+ Th17 cell cultures, $1,25(OH)_2D_3$ was not sufficient to induce IL-4 production. IL-10 production was not significantly increased by $1,25(OH)_2D_3$ in memory CCR6− and CCR6+ Th17 cell cultures (figure 1B). TNFα is expressed by activated T cells, and $1,25(OH)_2D_3$ suppressed TNFα expression in memory CCR6− and CCR6+ Th17 cells, but not in naïve T cells (figure 1B).

These data show that $1,25(OH)_2D_3$ has direct suppressive effects on the proinflammatory cytokines, IL-17A, IL-22, IFNγ and TNFα, in primary CCR6+ Th17 cell cultures, without affecting IL-4 and IL-10 cytokine production.

In Th17–RASF cocultures, $1,25(OH)_2D_3$ inhibited autocrine IL-17A production and induced an anti-inflammatory cytokine profile

We recently reported that, upon coculture with RASFs, Th17 cells induce a proinflammatory feedback loop, resulting in increased IL-17A production.[17] Interestingly, in the presence of $1,25(OH)_2D_3$, this proinflammatory loop was inhibited, as both the percentage of IL-17A-producing cells and IL-17A expression levels in Th17–RASF cocultures were significantly lower in the presence of $1,25(OH)_2D_3$ (~4.1-fold and ~1.8-fold, respectively, figure 2A,B). Moreover, $1,25(OH)_2D_3$ significantly inhibited the fraction of IL-22- and IFNγ-producing Th17 cells and IL-22 and IFNγ production in Th17–RASF cocultures (figure 2A,B). Of note, $1,25(OH)_2D_3$ significantly induced IL-4 and IL-10 expression in Th17–RASF cocultures, while no effects were observed for TNFα expression (figure 2B).

In summary, in Th17–RASF cocultures, $1,25(OH)_2D_3$ reduces Th17-associated proinflammatory cytokine production and induces IL-4 and IL-10 production.

$1,25(OH)_2D_3$ suppressed Th17-induced expression of both inflammatory cytokines and mediators of joint destruction by RASFs

Upon interaction with Th17 cells, RASFs are activated and the expression of IL-6, IL-8, MMP-1 and MMP-3 increased.[17] In Th17–RASF cocultures, $1,25(OH)_2D_3$ significantly reduced IL-6, IL-8 and MMP-1 expression (figure 3A). To verify, whether these $1,25(OH)_2D_3$-induced effects were mediated directly via Th17 cells, rather than direct effects on RASFs, we analysed IL-6, IL-8, MMP-1 and MMP-3 expression in RASF cultures with or without $1,25(OH)_2D_3$. In these unstimulated RASF monocultures, no effects of $1,25-(OH)_2D_3$ were found on IL-6, IL-8, MMP-1 and MMP-3 expression (figure 3B). However, in TNFα-stimulated RASF monocultures, inhibitory effects of $1,25-(OH)_2D_3$ were found on IL-6 and IL-8, but not on IL-22, MMP-1 and MMP-3 expression (figure 3B).

From this, we concluded that, in Th17–RASF cocultures, $1,25(OH)_2D_3$ inhibits IL-6, IL-8, IL-17A and MMP-1 expression.

Additive effect of $1,25(OH)_2D_3$ treatment combined with TNF blockade in Th17–RASF cocultures

Recently, we have shown that both IL-17A and TNFα are produced by Th17 cells and that these cytokines are involved in inducing a proinflammatory loop in Th17–RASF cocultures.[17] In contrast with $1,25(OH)_2D_3$, TNFα blockade did not influence IL-17A, IL-22 and IFNγ expression in the Th17–RASF cocultures (figure 4). This prompted us to investigate whether combining $1,25(OH)_2D_3$ and TNFα blockade could have an additional effect over TNFα blockade alone in Th17–RASF cocultures. TNFα blockade alone resulted in a significant reduction in IL-6, IL-8, MMP-1 and MMP-3 compared with the control situation. Combining $1,25(OH)_2D_3$ and TNFα blockade had a significant additional effect on IL-6, IL-8, MMP-1 and MMP-3 suppression, when compared with TNFα blockade alone (figure 4).

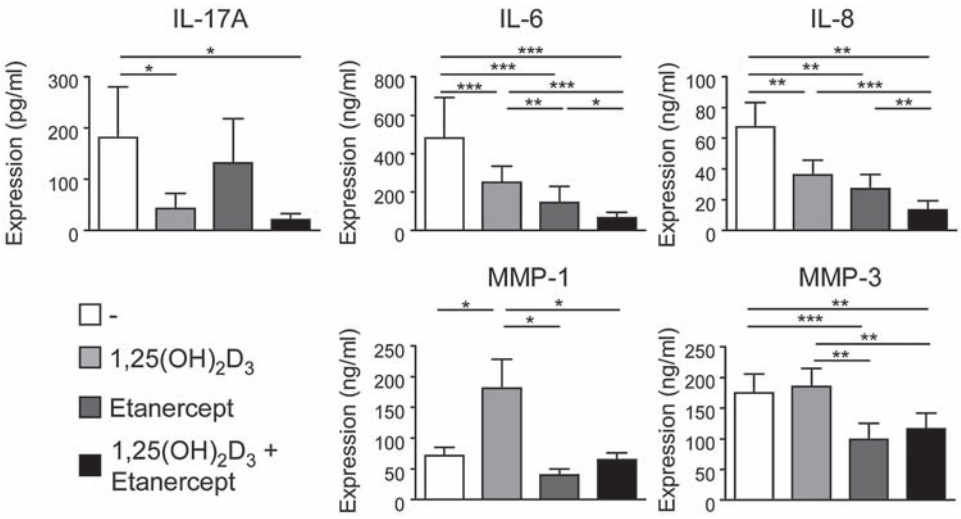

Figure 5 Additional effects on the suppression of interleukin (IL)-6 and IL-8 in synovial biopsy cultures in the presence of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and tumour necrosis factor α (TNFα) blockade compared with TNFα blockade alone. Synovial biopsy samples obtained from 14 patients with established rheumatoid arthritis were stimulated with αCD3/αCD28 and cultured with or without $1,25(OH)_2D_3$ and/or TNFα blockade. Expression of the indicated cytokines and matrix metalloproteases (MMPs) was detected in supernatant with ELISA. Mean and SEM are given. *p<0.05; **p<0.01; ***p<0.01, by two-sided Wilcoxon signed-rank test.

These findings show that, in Th17–RASF cocultures, TNF blockade does not suppress Th1 and Th17 cytokines, which can be overcome by $1,25(OH)_2D_3$. The combination of neutralising TNFα and $1,25(OH)_2D_3$ controls human Th17 activity and additively inhibits synovial inflammation.

Additional effect of $1,25(OH)_2D_3$ over TNF blockade in suppressing IL-6 and IL-8 expression in cultures of RA synovial tissue

To investigate the functional relevance of $1,25(OH)_2D_3$ effects in the Th17–RASF coculture system, the effects of $1,25(OH)_2D_3$ on synovial tissue of patients with established RA were analysed. Therefore synovial biopsy samples were cultured with αCD3/αCD28 and/or $1,25(OH)_2D_3$ and/or TNFα blockade. As we observed in the Th17–RASF cocultures, $1,25(OH)_2D_3$, but not TNFα blockade, significantly reduced the expression of IL-17A (figure 5). Moreover, both $1,25(OH)_2D_3$ and TNFα blockade resulted in significant suppression of IL-6 and IL-8 expression. Importantly, combining $1,25(OH)_2D_3$ with TNFα blockade had an additive effect compared with TNFα blockade alone in the inhibition of IL-6 and IL-8 expression (figure 5). In contrast, $1,25(OH)_2D_3$ had no effects on MMP-3 expression, and increased MMP-1 expression levels were observed. Consequently, no additional effects of $1,25(OH)_2D_3$ over TNFα blockade were found in the inhibition of MMP-1 and MMP-3 expression. However, combining $1,25(OH)_2D_3$ with TNFα blockade neutralised the $1,25(OH)_2D_3$ effect on MMP-1 induction (figure 5).

In summary, these findings show that $1,25(OH)_2D_3$ suppresses the expression of IL-6 and IL-8 and autocrine IL-17A expression in RA synovial tissue cultures. Combining $1,25(OH)_2D_3$ and TNFα blockade had additional value over TNFα blockade alone in further reducing IL-6 and IL-8 expression.

DISCUSSION

In this study, we found that $1,25(OH)_2D_3$, in contrast with TNFα blockade, directly regulates Th17 cytokine expression. Furthermore, $1,25(OH)_2D_3$ in combination with TNFα blockade is essential to fully neutralise pathological Th17 activity in RA synovial inflammation.

Evidence is accumulating that $1,25(OH)_2D_3$ has a suppressive role in murine experimental autoimmune models[32 33] and in human autoimmune diseases such as RA.[34–37] However, the mechanism underlying these suppressive effects is not fully elucidated. To analyse the effects of $1,25(OH)_2D_3$ on Th17 cytokine expression and activity, we have used different culturing approaches, including primary Th17 monocultures, Th17–RASF cocultures and synovial biopsy cultures. In all approaches, $1,25(OH)_2D_3$ treatment resulted in suppression of the proinflammatory cytokines, IL-17A, IL-22 and IFNγ. Moreover, the $1,25(OH)_2D_3$ effects in synovial tissue cultures not only show the relevance of the Th17–RASF cocultures as a functional T cell test system in RA, but importantly also show the therapeutic potential of $1,25(OH)_2D_3$/TNF blockade combination as a treatment to control synovial inflammation in RA, in particular in the presence of IL-17/Th17 activity.

In contrast, IL-4 and IL-10 production was not affected by 1,25(OH)2D3 in Th17 cultures, but was enhanced in Th17–RASF cocultures after 1,25(OH)2D3 treatment. These findings implicate that, in the coculture system, $1,25(OH)_2D_3$ supported Th2 function and/or inhibited factors that can negatively regulate IL-4 expression, such as IFNγ and T-Bet. On the other hand, in this study no effects were found of $1,25(OH)_2D_3$ treatment on TNFα production by Th17–RASF cocultures, whereas, in Th17 monocultures, TNFα production was significantly lower after $1,25(OH)_2D_3$ treatment. This suggests that, besides direct effects of $1,25(OH)_2D_3$ on Th17 cytokines, other factors that are induced in Th17–RASF cocultures and in the inflamed RA synovium are involved in the regulation of IL-4 and TNFα. We found no upregulation of Foxp3 expression in our Th17–RASF cocultures (data not shown), which indicates that the observed suppression is independent of T regulatory cell activity. Moreover, it has to be taken into account that the CCR6+ Th17 population is a heterogeneous population in which cells are present that are negative and positive for the production of either IL-17A or IFNγ or both. The molecular mechanism responsible for the induction of IL-4 by $1,25(OH)_2D_3$ in CCR6+ Th17 cocultures is at present under investigation.

$1,25(OH)_2D_3$ suppressed Th17 activity as observed by lower IL-6, IL-8 and MMP-1 levels in Th17–RASF cocultures. In an earlier study, we showed that both IL-17A and TNFα blockade are required to neutralise Th17 activity in Th17–RASF cocultures.[17] These cytokines were shown to have synergistic effects on fibroblast activation.[38] Since TNFα blockade had limited effects on the Th17 cytokines, IL-17A and IL-22, and $1,25(OH)_2D_3$ had limited effects on TNFα expression, the combination of TNFα blockade and $1,25(OH)_2D_3$ additionally downregulated Th17 activity. This indicates more valuable therapeutic potential of VDR signalling activation over the current TNF neutralisation strategies in patients with RA.

In contrast with Th17–RASF cocultures, $1,25(OH)_2D_3$ treatment resulted in an increase in MMP-1 expression in synovial biopsy cultures, which was overcome by the combination $1,25(OH)_2D_3$ and TNFα blockade. These different effects on MMP-1 expression in the synovial biopsy cultures compared with the Th17–RASF cocultures is still not well understood, but may be affected by the composition of the synovial biopsy samples. Besides T cells and synovial fibroblasts, these consist of other cells, such as inflammatory CD68+ macrophages. Of note, when TNF-stimulated RASFs were used, no stimulatory effect on MMP-1 expression by $1,25(OH)_2D_3$ was found. In line with our study, articular chondrocyte cultures, but not RASF cultures, have been shown to increase MMP-1 expression after $1,25(OH)_2D_3$ treatment.[39] Further experiments are needed to explain the phenomenon of increased MMP-1 expression by $1,25(OH)_2D_3$ in synovial tissue, focusing on the cellular composition and cellular interaction between cells present in the inflamed synovium.

Progression of joint damage and disease activity has been shown to correlate with the levels of IL-17A and Th17 cells in patients with RA.[16 40] The present study shows that the presence of IL-17A in the synovial biopsy samples has a marked influence on the outcome and demonstrates the additional value of the combination of TNFα blocking and $1,25(OH)_2D_3$ treatment compared with TNFα blocking alone. The more IL-17A produced by the synovial biopsy samples after T cell receptor and costimulatory activation, the more valuable the therapeutic potential effect on synovial inflammation attained by adding $1,25(OH)_2D_3$ to TNFα blocking. This suggests less effective anti-TNF therapy in patients with RA under increased IL-17A levels in these patients. In addition, we recently showed raised levels of memory Th17 cells in treatment-naïve patients with early RA compared with healthy controls.[17] Therefore together these data support the development of a clinical trial combining TNF blockade with VDR activation in patients with RA, in particular at the early stage of the disease.

TNFα blockade is a commonly used treatment in RA.[2] However, a large fraction (~20–40%) of patients do not respond to this therapy.[41] The present study shows that TNFα blockade has no effect on expression of Th17 cytokines such as IL-17A. This may explain why TNFα blockade alone is not effective in all patients with RA and is in line with the suggestion based on preliminary data that TNFα blockade may be less effective in patients with RA who have especially raised levels of IL-17A.[5] Therefore it would be of great interest to investigate whether the group of anti-TNF non-responders has raised levels of Th17 cytokines and/or Th17 cell activity. This study further shows that, for full neutralisation of Th17 activity in RA synovial inflammation, TNFα blockade needs to be combined with $1,25(OH)_2D_3$ signalling. This underlines the importance of finding combinations of therapeutic approaches to improve the efficacy of current treatment strategies for RA, such as TNFα blockade.

Currently, there is considerable interest in targeting IL-17A or Th17 cells in the treatment of RA and other Th17-mediated disorders.[4 8] In line with this, activation of VDR signalling might be a contending approach. In relation to IL-17A blockade alone, activation of VDR signalling has the advantage that, in addition to IL-17A, other Th17 cytokines, such as IL-17F and IL-22, are downregulated, whereas cytokines such as IL-4 and IL-10 are induced (present study).[27] However, activation of VDR signalling with $1,25(OH)_2D_3$ can have severe side effects such as hypercalcaemia.[42] For this reason, future research should focus on the identification of $1,25(OH)_2D_3$ targets in T cells, and Th17 cells in particular, which may have therapeutic potential for the treatment of RA. Moreover, it should be taken into account that cells of the immune system such as macrophages express the vitamin D-converting enzyme, 1α-hydroxylase, enabling the conversion of $25(OH)D_3$ into $1,25(OH)_2D_3$.[24 43 44] This may imply increased local levels of $1,25(OH)_2D_3$ after administration of $25(OH)D_3$.

In conclusion, this study shows direct suppressive effects of $1,25(OH)_2D_3$, in contrast with TNFα blockade, on Th17 cytokine expression and activity by Th17 cells from patients with RA. This implies that adding activation of VDR signalling to current TNF blockade therapy may have an additional role in fully neutralising pathogenic Th17 activity in RA and other Th17-mediated autoimmune diseases. Moreover, our data suggest less effective anti-TNF therapy in patients with RA with increased IL-17A levels and provide a rationale for a therapeutic trial combining TNF blockade with VDR activation in patients with RA and potentially also in other Th17-mediated autoimmune disorders.

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TNF blockade requires 1,25(OH)2D3 to control human Th17-mediated synovial inflammation

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