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1α,25-dihydroxyvitamin D_3 inhibits matrix metalloproteinases induced by *Mycobacterium tuberculosis* infection

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Summary

Matrix metalloproteinases (MMP) can degrade all components of pulmonary extracellular matrix. *Mycobacterium tuberculosis* induces production of a number of these enzymes by human macrophages, and these are implicated in the pathogenesis of pulmonary cavitation in tuberculosis. The active metabolite of vitamin D, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], has previously been reported to inhibit secretion of MMP-9 in human monocytes (MN), but its influence on the secretion and gene expression of MMP and tissue inhibitors of MMP (TIMP) in *M. tuberculosis*-infected cells has not previously been investigated. We therefore determined the effects of 1α,25$(OH)_2D_3$ on expression, secretion and activity of a number of MMP and TIMP in *M. tuberculosis*-infected human leucocytes; we also investigated the effect of 1α,25$(OH)_2D_3$ on the secretion of interleukin-10 (IL-10) and prostaglandin E_2 (PGE_2), both transcriptional regulators of MMP expression. We found that *M. tuberculosis* induced expression of *MMP-1*, *MMP-7* and *MMP-10* in MN and *MMP-1* and *MMP-10* in peripheral blood mononuclear cells (PBMC). 1α,25$(OH)_2D_3$ significantly attenuated *M. tuberculosis*-induced increases in expression of *MMP-7* and *MMP-10*, and suppressed secretion of MMP-7 by *M. tuberculosis*-infected PBMC. *MMP-9* gene expression, secretion and activity were significantly inhibited by 1α,25$(OH)_2D_3$ irrespective of infection. In contrast, the effects of 1α,25$(OH)_2D_3$ on the expression of *TIMP-1*, *TIMP-2* and *TIMP-3* and secretion of TIMP-1 and TIMP-2 were small and variable. 1α,25$(OH)_2D_3$ also induced secretion of IL-10 and PGE_2 from *M. tuberculosis*-infected PBMC. These findings represent a novel immunomodulatory role for 1α,25$(OH)_2D_3$ in *M. tuberculosis* infection.

Keywords: interleukin-10; matrix metalloproteinases; mycobacteria; TB; tissue inhibitors of matrix metalloproteinases; vitamin D

Introduction

Tuberculosis (TB) is the world's leading bacterial cause of death.[1] The ability of *Mycobacterium tuberculosis* to induce degradation of pulmonary extracellular matrix contributes to its success as a pathogen: induction of cavitation allows bacilli to replicate in an immunologically privileged site, promoting persistence and transmission, while penetration of the alveolar basement membrane allows extra-pulmonary dissemination of infection. Matrix metalloproteinases (MMP), a family of zinc- and calcium-dependent endopeptidases, are capable of degrading all components of pulmonary extracellular matrix. Generally, MMP are not expressed in healthy non-calcified tissues, but they are upregulated in activated cells where their primary role is to facilitate tissue remodelling and repair. They also regulate the innate immune response by controlling cytokine and chemokine processing, apoptosis and antimicrobial peptide activation (for review see ref. 2) Excess MMP activity in response to *M. tuberculosis*

infection may therefore lead to excessive tissue degradation and, ultimately, pulmonary cavitation.

There are 24 known mammalian MMP which possess broad and overlapping specificities. They are expressed by a wide variety of cells, including lymphocytes, resting MN and activated macrophages. MMP-1 (interstitial collagenase) and MMP-9 (92 000 molecular weight gelatinase B) are the major secreted MMP of human MN and alveolar macrophages under basal conditions and on stimulation with lipopolysaccharide, phorbol 12-myristate 13-acetate or concanavalin A.[3] *Mycobacterium tuberculosis* induces the expression of *MMP-1*, *MMP-7* and *MMP-10* in human macrophages[4] and increased expression of *MMP-1*, *MMP-7* and *MMP-9* has also been demonstrated in cells isolated from the lungs of TB patients, with MMP-1 and MMP-7 colocalizing to macrophages around the central area of necrosis in tuberculous granulomata.[4,5] Furthermore, circulating concentrations of MMP-9 have also been shown to correlate with the severity of pulmonary TB.[6]

The catalytic activity of MMP is primarily transcriptionally regulated, with a more delicate control achieved via pro-enzyme proteolytic activation and enzyme inhibition. Tissue inhibitors of matrix metalloproteinases (TIMP) are the major inhibitors of MMP activity; they function by binding the MMP catalytic site with 1 : 1 stoichiometry.[7,8] The four TIMP that have been identified share broad specificities and show constitutive expression in a variety of cells. Expression of *TIMP-2* and *TIMP-3* has been shown to decrease during *M. tuberculosis* infection of human macrophages, while *TIMP-1* expression is suppressed by *M. tuberculosis* in human pulmonary epithelial cells.[4,9]

The regulation of MMP and TIMP expression is complex and not yet fully understood. The anti-inflammatory cytokine interleukin-10 (IL-10) has previously been reported to inhibit mononuclear phagocyte MMP-1, MMP-7 and MMP-9 secretion and to induce *TIMP-1* expression.[10,11] The eicosanoid inflammatory mediator prostaglandin E_2 (PGE_2) also regulates MMP expression and secretion: in mononuclear phagocytes, secretion of MMP-1 and MMP-7 is PGE_2-dependent,[4,11,12] while in cocultures of MN and fibroblasts, PGE_2 suppresses the secretion and activation of MMP-1.[13]

The active metabolite of vitamin D, 1α,25-dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$], has previously been reported to inhibit both basal and staphylococcus-stimulated production of MMP-9, but not MMP-1, in human MN and alveolar macrophages.[14] $1\alpha,25(OH)_2D_3$ also upregulates the IL-10 receptor,[15] induces IL-10 synthesis[16,17] and modulates gene expression of inducible prostaglandin H synthase-2 (PGHS-2, a rate-limiting enzyme in PGE_2 synthesis) and type 1 15-hydroxyprostaglandin dehydrogenase (15-PGDH, the key enzyme in PGE_2 catabolism) *in vitro*.[18,19] Specific affinity receptors for $1\alpha,25(OH)_2D_3$ are present on a number of leucocytes, including resting MN, macrophages and T and B lymphocytes.[20,21] Furthermore, it has recently been shown that synthesis of $1\alpha,25(OH)_2D_3$ may occur in tuberculous granulomata as a consequence of *M. tuberculosis*-induced upregulation of CYP27B1, the enzyme that converts 25-hydroxyvitamin D_3 to $1\alpha,25(OH)_2D_3$.[22] Reports that interactions between T cells and mononuclear phagocytes enhance MMP expression in both cell types[11,22–25] provide a rationale for investigating MMP regulation in peripheral blood mononuclear cells (PBMC) as well as MN. We therefore investigated whether $1\alpha,25(OH)_2D_3$ modulates expression, secretion or activity of MMP-1, MMP-7, MMP-9 and MMP-10 in *M. tuberculosis*-infected PBMC and MN, and whether the changes observed were associated with changes in the expression or secretion of TIMP-1, TIMP-2, TIMP-3, IL-10, PGHS-2, 15-PGDH and PGE_2.

Materials and methods

Culture of M. tuberculosis

Mycobacterium tuberculosis H37Rv was grown to mid-log phase in Middlebrook 7H9 broth supplemented with 10% albumin dextrose catalase (Difco, Detroit, MI, USA) and 0·04% Tween 80 (Sigma, St Louis, MO, USA). An equal volume of 30% glycerol was added, and aliquots of the resulting suspension of bacilli were placed into vials. Colony forming units (CFU) per millilitre for each batch of aliquots was determined by plating serial dilutions onto 7H11 agar before freezing at −80°. Vials were defrosted immediately before tissue culture experiments, and knowledge of CFU/ml determined at the time of freezing was used to calculate the volume of inoculum necessary to achieve the desired multiplicity of infection (MOI). For each inoculum the CFU/ml was also determined by plating onto 7H11 agar at the time of infection to confirm that MOI was correct.

Culture of PBMC and MN with $1\alpha,25(OH)_2D_3$ *and* M. tuberculosis

The PBMC were isolated from buffy coats of healthy blood donors over Ficoll, and MN preparations were obtained by adherence as previously described.[26,27] For initial experiments to determine the influence of $1\alpha,25(OH)_2D_3$ and *M. tuberculosis* on messenger RNA (mRNA) levels, cells from 10 donors were plated in six-well plates at 5×10^6 PBMC/MN in 2 ml RPMI 1640/10% fetal calf serum and incubated with either 10^{-6} M $1\alpha,25(OH)_2D_3$ dissolved in ethanol (0·1% final concentration) or 0·1% ethanol alone for 72 hr. Cells were then infected with *M. tuberculosis* H37Rv. For MN, the MOI bacillus : mononuclear phagocyte was 1 : 1; non-phagocytosed bacilli were washed off 4 hr postinfection. For PBMC, MOI was normalized to the average MN count

(10% PBMC), giving MOI bacillus : PBMC 0·1 : 1. Infected cells were cultured in the continued presence or absence of $1\alpha,25(OH)_2D_3$ for a further 96 hr. Supernatants were harvested immediately before infection (for determination of constitutive cytokine and MMP secretion), at 24 hr postinfection (for determination of PGE_2 concentration) and at 96 hr postinfection (for determination of IL-10 concentration). Supernatants from *M. tuberculosis*-infected cell culture were sterilized with polysulphone filters (Whatman, Brentford, UK). For subsequent experiments to determine the influence of $1\alpha,25(OH)_2D_3$ and *M. tuberculosis* on supernatant concentration and activity of MMP, PBMC from six donors were plated in 48-well plates at $2{\cdot}5 \times 10^6$ PBMC in 1 ml RPMI-1640/10% fetal calf serum and incubated with either 0·1% ethanol, 10^{-6} M $1\alpha,25(OH)_2D_3$ dissolved in ethanol (0·1% final concentration), *M. tuberculosis*, or both *M. tuberculosis* and $1\alpha,25(OH)_2D_3$. The MOI bacillus : PBMC was 0·1 : 1. Supernatants were harvested after 72 hr incubation, and sterilized with polysulphone filters (Whatman).

RNA extraction and quantitative reverse transcription–polymerase chain reaction

RNA samples were extracted immediately following isolation of PBMC/MN (0 hr), immediately before infection (72 hr) and at 6 hr and 24 hr postinfection (78 hr and 96 hr post-seeding respectively). At each time-point, supernatants were aspirated and the cell monolayer was immediately lysed and shredded using the RNeasy extraction kit (Qiagen, Valencia, CA). RNA was reverse transcribed using the Quantitect reverse transcription kit (Qiagen) that includes a DNase digest step. Complementary DNA was used in quantitative polymerase chain reaction for *MMP-1*, *MMP-7*, *MMP-9*, *MMP-10*, *TIMP-1*, *TIMP-2*, *TIMP-3*, *IL-10*, *PGHS-2*, *15-PGDH* and β-actin on the ABI Prism 7000 platform. Primers and probes were obtained as predeveloped assay reagents (Applied Biosystems, Foster City, CA) with the exception of β-actin, for which primer and probe sequences were as follows: forward primer: CCT GGCACCCAGCACAAT; reverse primer: GCCGATCCACACGGAGTACT; probe: 5′-VIC-ATCAAGATCATTGCTCCTCCTGAGCGC-BQ.

Each reaction was multiplexed by, and normalized to, the β-actin content. Fold induction over unseeded cell samples (0 hr) was calculated by the $\Delta\Delta$CT method as described elsewhere (User Bulletin #2, available for download from http://www.appliedbiosystems.com).

Enzyme-linked immunosorbent assay

Supernatant concentrations of MMP-1, MMP-7, MMP-9, MMP-10, TIMP-1, TIMP-2, IL-10 and PGE_2 were determined by enzyme-linked immunosorbent assay using kits purchased from the following manufacturers: R&D Systems, Abingdon, UK: IL-10 (sensitivity, 27 pg/ml), total MMP-7 (0·2 ng/ml), total MMP-9 (9·7 ng/ml), total TIMP-1 (1·2 ng/ml) and PGE_2 (58 pg/ml); Amersham Biosciences, Little Chalfont, UK: total MMP-1 (2·4 ng/ml); and RayBiotech, Norcross, GA: total MMP-10 (1·5 pg/ml), and TIMP-2 (10 pg/ml).

Enzyme activity assays

Supernatant activities of MMP-1 and MMP-9 were determined with colorimetric assays using modified pro-urokinase (Amersham Biosciences).[28] Supernatant activity of MMP-7 was measured with a fluorimetric assay using 5-FAM labelled FRET peptide substrates (Anaspec, San Jose, CA). No kits for MMP-10 secretion were available with sufficient sensitivity.

Statistical analysis

RNA fold induction values were normalized by $\log_{10}$ transformation before statistical analysis. Gaussian distribution was confirmed using the D'Agostino and Pearson test. Statistical significance was tested using repeated measures analysis of variance with Bonferroni *post hoc* correction applied to tested comparisons (significance level, $P < 0{\cdot}05$).

Results

$1\alpha,25(OH)_2D_3$ inhibits constitutive expression, but not activity, of MMP

The mRNA levels of four MMP previously reported to be regulated by *M. tuberculosis* infection were analysed in human PBMC and MN cultured for 72 hr in the presence or absence of $1\alpha,25(OH)_2D_3$ (Fig. 1). In the absence of $1\alpha,25(OH)_2D_3$ an increase in gene expression of *MMP-7* (664-fold in PBMC and sevenfold in MN, $P < 0{\cdot}01$), *MMP-9* (191-fold in PBMC and 22-fold in MN, $P < 0{\cdot}001$) and *MMP-10* (10-fold in PBMC, $P < 0{\cdot}01$) was observed; no change in expression of *MMP-1* was seen. Treatment with $1\alpha,25(OH)_2D_3$ inhibited constitutive expression of *MMP-9* (40-fold in PBMC and 25-fold in MN, $P < 0{\cdot}001$) and *MMP-10* (12-fold in PBMC and 10-fold in MN, $P < 0{\cdot}001$) but did not influence constitutive expression of *MMP-1* or *MMP-7*. Concentrations of MMP were also determined in supernatants harvested after 72 hr of culture (Fig. 2): $1\alpha,25(OH)_2D_3$ suppressed the secretion of MMP-9 in PBMC (14-fold suppression, $P < 0{\cdot}001$) but did not influence the secretion of MMP-7 or MMP-10. MMP-1 was undetectable in 10 of 10 $1\alpha,25(OH)_2D_3$-treated samples versus 8 of 10 untreated samples. Activity of MMP-1, MMP-7 and MMP-9 in these supernatants was also assayed (Fig. 3):

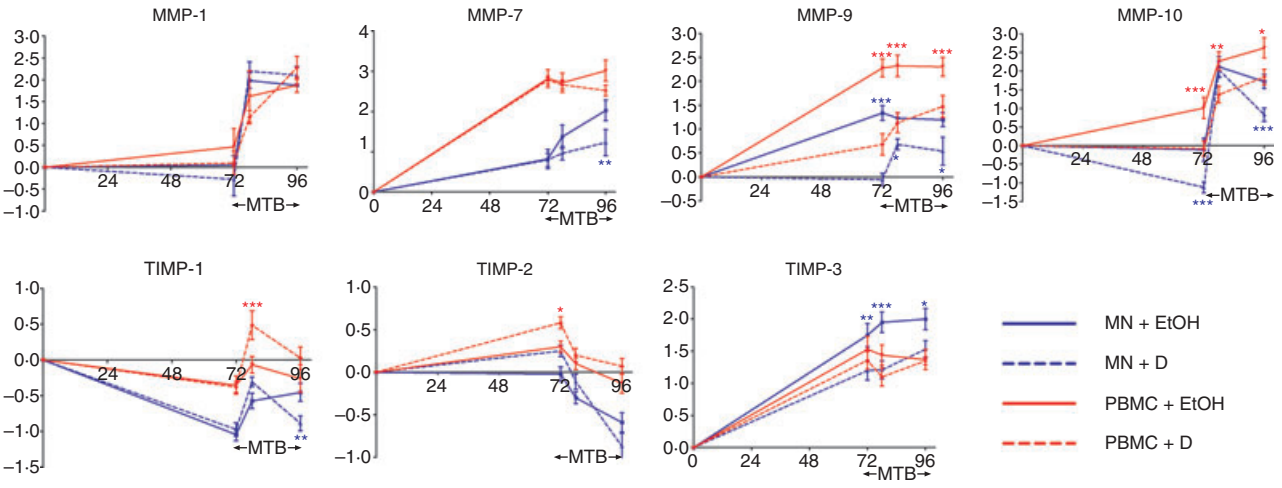

Figure 1. Influence of 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$; D] and *Mycobacterium tuberculosis* (MTB) on gene expression of matrix metalloproteinases (MMP) and tissue inhibitors of matrix metalloproteinases (TIMP) in peripheral blood mononuclear cells (PBMC) and monocytes (MN). 1α,25$(OH)_2D_3$ suppressed expression of *MMP-9* and *MMP-10* in uninfected cells, while *M. tuberculosis* increased expression of *MMP-1*, *MMP-7* and *MMP-10* in untreated cells. When 1α,25$(OH)_2D_3$-treated cells were infected with *M. tuberculosis*, preinfection decreases in expression of *MMP-9* and *MMP-10* were maintained during infection; 1α,25$(OH)_2D_3$ also suppressed MN expression of *MMP-7* in an infection-specific manner. In contrast, the effects of 1α,25$(OH)_2D_3$ and *M. tuberculosis* on *TIMP* gene expression were small and variable. Ten donors; error bars, SE; *x*-axis, hours postseeding; *y*-axis, mean $\log_{10}$ fold induction; SE; $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$; EtOH = 0·1% ethanol vehicle.

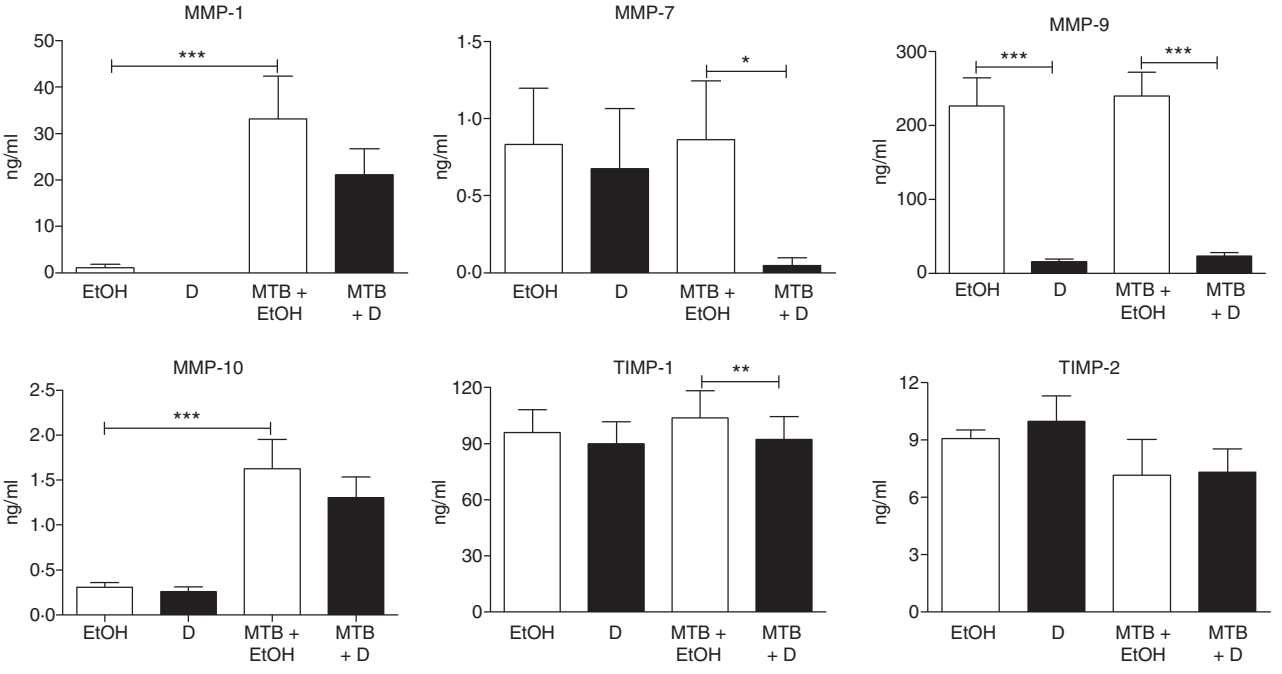

Figure 2. Influence of 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$; D] and *Mycobacterium tuberculosis* (MTB) on secretion of matrix metalloproteinases (MMP) and tissue inhibitors of matrix metalloproteinases (TIMP) by peripheral blood mononuclear cells (PBMC). 1α,25$(OH)_2D_3$ suppressed secretion of MMP-9 in uninfected cells, while *M. tuberculosis* stimulated secretion of MMP-1 and MMP-10 in untreated cells. Treatment with 1α,25$(OH)_2D_3$ induced biologically significant suppression of secretion of MMP-7 and MMP-9 in *M. tuberculosis*-infected cells. Six donors; error bars, SE; $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$; EtOH = 0·1% ethanol vehicle.

1α,25$(OH)_2D_3$ did not influence the activity of MMP-1 or MMP-9, and MMP-7 activity was undetectable in all samples.

Three regulators of MMP activity, TIMP-1, TIMP-2 and TIMP-3, were also analysed to determine the effect of 1α,25$(OH)_2D_3$ on their constitutive expression. *In vitro*

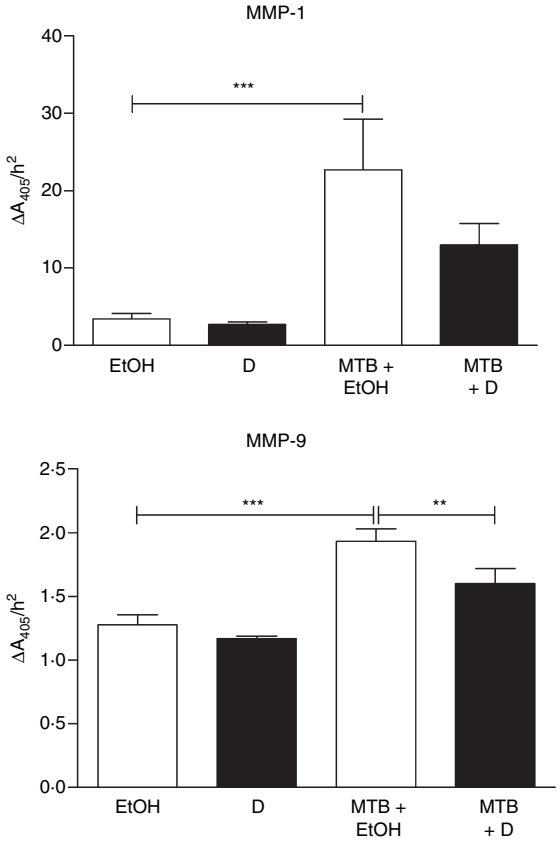

Figure 3. Influence of 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$; D] and *Mycobacterium tuberculosis* (MTB) on matrix metalloproteinase (MMP) activity of peripheral blood mononuclear cell supernatants. 1α,25$(OH)_2D_3$ did not influence activity of MMP-1 or MMP-9 in uninfected cells. *Mycobacterium tuberculosis* significantly induced activity of MMP-1 and MMP-9, and this effect was significantly attenuated by treatment with 1α,25$(OH)_2D_3$ for MMP-9. Six donors; error bars, SE; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$; EtOH = 0·1% ethanol vehicle.

culture had a variable effect on TIMP expression: *TIMP-1* expression decreased 11-fold in MN ($P < 0{\cdot}001$) while *TIMP-2* expression increased twofold in PBMC ($P < 0{\cdot}05$) and *TIMP-3* expression increased 34-fold in PBMC and 56-fold in MN ($P < 0{\cdot}001$) (Fig. 1). 1α,25(OH)2D3 induced a twofold increase in *TIMP-2* expression in PBMC ($P < 0{\cdot}05$) and a fourfold decrease in *TIMP-3* expression in MN ($P < 0{\cdot}01$) (Fig. 1) but did not influence expression of *TIMP-1* or secretion of TIMP-1 or TIMP-2 (Fig. 2).

Mycobacterium tuberculosis induces MMP expression, secretion and activity

Cells cultured for 72 hr with and without 1α,25$(OH)_2D_3$ were infected with *M. tuberculosis* H37Rv, and MMP and TIMP mRNA levels were determined at 6 and 24 hr postinfection. The end-point observations are summarized in Table 1. *Mycobacterium tuberculosis* upregulated gene expression of *MMP-1* (26-fold at 24 hr in PBMC, $P < 0{\cdot}05$; 103-fold at 6 hr and 57-fold at 24 hr in MN, $P < 0{\cdot}001$), *MMP-7* (16-fold at 24 hr in MN, $P < 0{\cdot}001$) and *MMP-10* (18-fold at 6 hr and 41-fold at 24 hr in PBMC, $P < 0{\cdot}001$; 170-fold at 6 hr and 68-fold at 24 hr in MN, $P < 0{\cdot}001$), but did not influence gene expression of *MMP-9* in either PBMC or MN. *Mycobacterium tuberculosis* induced secretion of MMP-1 (30-fold increase, $P < 0{\cdot}001$) and MMP-10 (fivefold increase, $P < 0{\cdot}001$) from PBMC at 72 hr postinfection but did not influence the secretion of MMP-7 or MMP-9 (Fig. 2). It also induced the activity of MMP-1 (sevenfold increase, $P < 0{\cdot}001$) and MMP-9 (1·5-fold increase, $P < 0{\cdot}001$) (Fig. 3). In contrast, the influence of *M. tuberculosis* infection on TIMP expression was small and variable: *M. tuberculosis* induced *TIMP-1* gene expression fourfold in MN at 24 hr postinfection ($P < 0{\cdot}001$), and suppressed TIMP-2 expression threefold in PBMC and fourfold in MN at 24 hr postinfection ($P < 0{\cdot}001$) but did not influence the expression of TIMP-3 or the secretion of TIMP-1 or TIMP-2 (Figs 1, 2).

1α,25$(OH)_2D_3$ inhibits *M. tuberculosis*-induced increases in MMP expression, secretion and activity

When 1α,25$(OH)_2D_3$-treated cells were infected with *M. tuberculosis*, the pretreatment decreases in mRNA level induced by 1α,25$(OH)_2D_3$ were maintained during infection for both *MMP-9* and *MMP-10* (for *MMP-9*, 16-fold decrease at 6 hr postinfection and sevenfold decrease at 24 hr postinfection in PBMC ($P < 0{\cdot}001$) and fourfold decrease at 6 hr postinfection and fivefold decrease at 24 hr postinfection in MN ($P < 0{\cdot}05$); for *MMP-10*, eightfold decrease at 6 hr postinfection ($P < 0{\cdot}01$) and sixfold decrease at 24 hr postinfection ($P < 0{\cdot}05$) in PBMC and eightfold decrease at 24 hr postinfection in MN, $P < 0{\cdot}001$) (Fig. 1). Additionally, 1α,25$(OH)_2D_3$ exerted an infection-specific effect on *MMP-7*, downregulating gene expression in MN sixfold at 24 hr postinfection ($P < 0{\cdot}01$). Preincubation with 1α,25$(OH)_2D_3$ also inhibited secretion of MMP-7 (17-fold, $P < 0{\cdot}05$) and MMP-9 (10-fold, $P < 0{\cdot}001$) from *M. tuberculosis*-infected PBMC (Fig. 2). A trend towards decreased secretion of MMP-1 and MMP-10 was also observed, but this did not attain statistical significance after correction for multiple analyses. A moderate decrease in activity of MMP-9 (1·2-fold, $P < 0{\cdot}01$) was also observed (Fig. 3). The effects of preincubation with 1α,25$(OH)_2D_3$ on TIMP expression and secretion in *M. tuberculosis*-infected cells were small and variable: 1α,25$(OH)_2D_3$ induced *TIMP-1* gene expression in *M. tuberculosis*-infected PBMC at 6 hr postinfection (fourfold increase, $P < 0{\cdot}001$), but

Table 1. Summary of significant ($P < 0{\cdot}05$) changes in gene expression following 1α,25-dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$] treatment and *Mycobacterium tuberculosis* infection of human peripheral blood mononuclear cells (PBMC) and monocytes (MN)

	$1\alpha,25(OH)_2D_3$ versus 0·1% ethanol		*M. tuberculosis* versus uninfected		*M. tuberculosis* + $1\alpha,25(OH)_2D_3$ versus *M. tuberculosis* + 0·1% ethanol	
Gene	PBMC	MN	PBMC	MN	PBMC	MN
MMP-1	–	–	↑↑	↑↑	–	–
MMP-7	–	–	–	↑↑	–	↓
MMP-9	↓↓	↓↓	–	–	↓↓	↓
MMP-10	↓↓	↓↓	↑↑	↑↑	↓	↓
TIMP-1	–	–	–	↑	↑	↓
TIMP-2	↑	–	↓	↓	–	–
TIMP-3	–	↓	–	–	–	↓
IL-10	–	–	–	↑↑	↑	↑
15-PGDH	↑	–	↑	↑	–	–
PGHS-2	↓	–	↑↑	↑↑	–	↑↑

↑, < 10-fold increase; ↑↑, ≥ 10-fold increase; ↓, < 10-fold decrease; ↓↓, ≥ 10-fold decrease; –, no significant effect.

IL-10, interleukin-10; MMP, matrix metalloproteinase; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; PGHS-2, prostaglandin H synthase-2; TIMP, tissue inhibitor of matrix metalloproteinase.

suppressed *TIMP-1* gene expression in MN at 24 hr postinfection (threefold decrease, $P < 0{\cdot}01$). $1\alpha,25(OH)_2D_3$ also suppressed expression of *TIMP-3* (sixfold decrease in MN at 6 hr postinfection, $P < 0{\cdot}001$, Fig. 1) but did not induce biologically significant changes in secretion of TIMP-1 or TIMP-2 by *M. tuberculosis*-infected PBMC (Fig. 2).

$1\alpha,25(OH)_2D_3$ induces IL-10 secretion in *M. tuberculosis*-infected PBMC

The regulatory cytokine IL-10 has previously been reported to modulate the expression of MMP and TIMP[10] and it is also $1\alpha,25(OH)_2D_3$-inducible.[16,17] We therefore proceeded to determine the effects of $1\alpha,25(OH)_2D_3$ and *M. tuberculosis* on IL-10 expression and secretion (Fig. 4). $1\alpha,25(OH)_2D_3$ had no significant effect on constitutive expression of IL-10 in PBMC or MN. *Mycobacterium tuberculosis* upregulated IL-10 mRNA levels in MN at 6 hr postinfection (11-fold increase, $P < 0{\cdot}001$), but not at 24 hr postinfection and not in PBMC at either time-point. $1\alpha,25(OH)_2D_3$ augmented the IL-10 response to *M. tuberculosis* in PBMC at 6 hr postinfection, causing a sixfold increase in gene expression that was maintained at 24 hr postinfection ($P < 0{\cdot}001$). This was reflected by a threefold increase in IL-10 secretion by *M. tuberculosis*-infected PBMC ($P < 0{\cdot}001$, Fig. 4). $1\alpha,25(OH)_2D_3$ induced a more modest increase in IL-10 expression in *M. tuberculosis*-infected MN (threefold increase at 24 hr post-infection, $P < 0{\cdot}01$) but did not induce a statistically significant increase in IL-10 secretion by *M. tuberculosis*-infected MN at 72 hr postinfection (Fig. 4).

$1\alpha,25(OH)_2D_3$ upregulates PGE_2 secretion in MN, both in the presence and absence of *M. tuberculosis* infection

As macrophage MMP expression is largely PGE_2-dependent[12,29] and because $1\alpha,25(OH)_2D_3$ has been shown to modulate mRNA levels of rate-limiting enzymes in PGE_2 synthesis and catabolism (PGHS-2 and 15-PGDH, respectively)[18,19] we investigated whether expression of these genes and secretion of PGE_2 were modulated by $1\alpha,25(OH)_2D_3$ and *M. tuberculosis* (Fig. 5). In the absence of $1\alpha,25(OH)_2D_3$ a significant decrease in expression of *15-PGDH* (fivefold in MN, $P < 0{\cdot}01$) and *PGHS-2* (27-fold in PBMC and 223-fold in MN, $P < 0{\cdot}001$) was observed. In PBMC, $1\alpha,25(OH)_2D_3$ attenuated this constitutive decrease in *15-PGDH* expression (fivefold increase, $P < 0{\cdot}01$), but induced a further decrease in *PGHS-2* expression (sevenfold decrease, $P < 0{\cdot}05$); $1\alpha,25(OH)_2D_3$ did not influence the expression of either gene in MN. *Mycobacterium tuberculosis* increased mRNA levels of *PGHS-2* in both PBMC (39-fold at 6 hr postinfection and 83-fold at 24 hr postinfection, $P < 0{\cdot}001$) and MN (1097-fold at 6 hr postinfection and 67-fold at 24 hr postinfection, $P < 0{\cdot}001$) and also induced expression of *15-PGDH* in PBMC at 6 hr postinfection (fourfold, $P < 0{\cdot}05$) and in MN at 24 hr postinfection (fivefold, $P < 0{\cdot}01$). $1\alpha,25(OH)_2D_3$ further increased *PGHS-2* mRNA levels in *M. tuberculosis*-infected MN at both 6 hr and 24 hr postinfection (fivefold and 15-fold increase respectively, $P < 0{\cdot}01$) but did not influence expression of *PGHS-2* in *M. tuberculosis*-infected PBMC or expression of

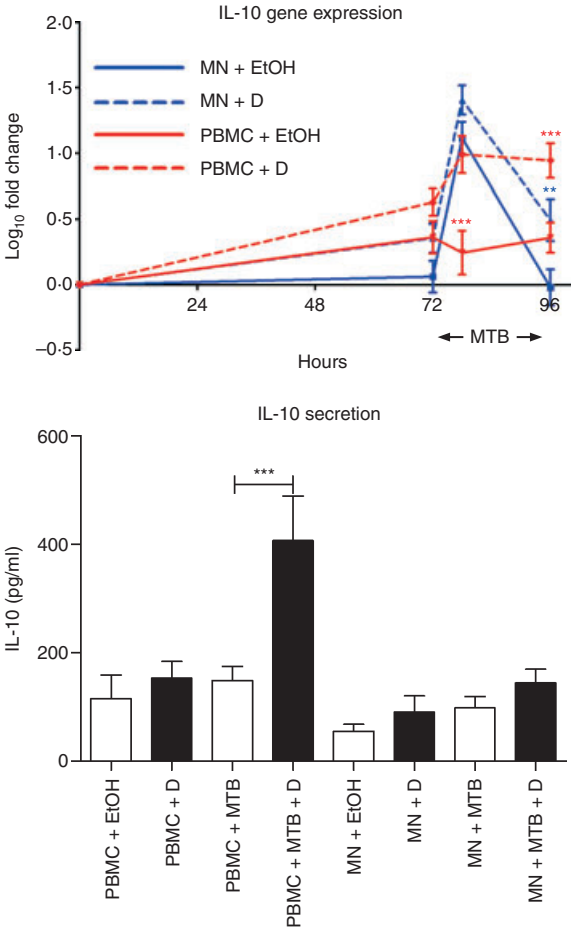

Figure 4. Influence of 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$; D] and *Mycobacterium tuberculosis* (MTB) on interleukin-10 (IL-10) gene expression and secretion by peripheral blood mononuclear cells (PBMC) and monocytes (MN). 1α,25$(OH)_2D_3$ had no effect on constitutive *IL-10* gene expression in PBMC or MN. *Mycobacterium tuberculosis* induced *IL-10* gene expression in MN at 6 hr postinfection. 1α,25$(OH)_2D_3$ induced *IL-10* gene expression in *M. tuberculosis*-infected PBMC and MN, and induced IL-10 secretion from PBMC in the presence, but not in the absence, of *M. tuberculosis*. Ten donors; error bars, SE; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$; EtOH = 0·1% ethanol vehicle.

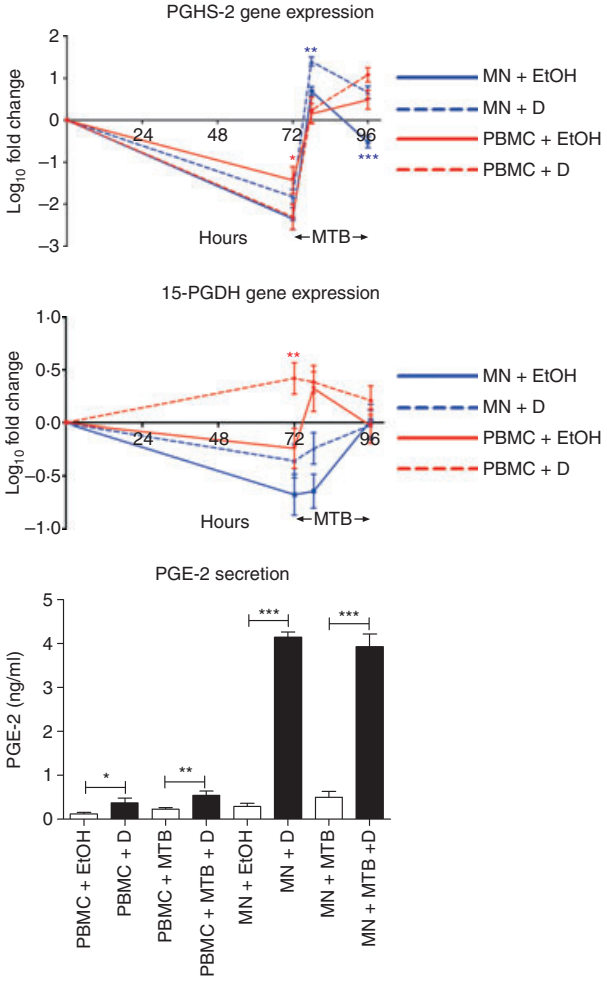

Figure 5. Influence of 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$; D] and *Mycobacterium tuberculosis* (MTB) on gene expression of prostaglandin H synthase-2 (PGHS-2) and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) and secretion of prostaglandin E_2 (PGE_2) by peripheral blood mononuclear cells (PBMC) and monocytes (MN). 1α,25$(OH)_2D_3$ induced constitutive expression of *15-PGDH* and suppressed constitutive expression of *PGHS-2* in PBMC. *M. tuberculosis* induced gene expression of *PGHS-2* (in both PBMC and MN) and *15-PGDH* (in MN). Treatment with 1α,25$(OH)_2D_3$ further upregulated *PGHS-2* gene expression in *M. tuberculosis*-infected MN but had no effect on *15-PGDH* expression in *M. tuberculosis*-infected cells. 1α,25$(OH)_2D_3$ induced secretion of PGE_2 in *M. tuberculosis*-uninfected and *M. tuberculosis*-infected PBMC and MN. Mean of 10 donors; error bars, SE. $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$; EtOH = 0·1% ethanol vehicle.

15-PGDH in *M. tuberculosis*-infected PBMC or MN. *Mycobacterium tuberculosis* did not influence the secretion of PGE_2 from PBMC or MN, but 1α,25$(OH)_2D_3$ stimulated the secretion of PGE_2 from all cells both in the absence and in the presence of *M. tuberculosis* (threefold in uninfected PBMC, twofold in *M. tuberculosis*-infected PBMC, 14-fold in uninfected MN and eightfold in *M. tuberculosis*-infected MN, $P < 0{\cdot}05$; Fig. 5).

Discussion

The MMP family of enzymes has been a therapeutic target for over 20 years, but dose-limiting side-effects of candidate agents have prevented any from entering clinical trials.[30] We have previously shown that the administration of large bolus doses of vitamin D is safe, and suppresses circulating concentrations of MMP-9[31] as well as modulating antimycobacterial immune responses.[32] We and others have also previously demonstrated that 1α,25$(OH)_2D_3$ induces the antimicrobial peptide cathelicidin LL-37, which possesses antituberculous activity.[22,33,34]

This report describes a complementary mechanism whereby vitamin D could exert a beneficial effect on host response to *M. tuberculosis* by inhibiting the expression, secretion and activity of a number of MMP induced by *M. tuberculosis*, consequently limiting the degradation of extracellular matrix and decreasing pulmonary cavitation to preserve lung function and reduce infectiousness.

Mononuclear phagocytes are the site of *M. tuberculosis* replication and containment, and the principal source of MMP. It has previously been shown that $1\alpha,25(OH)_2D_3$ regulates the secretion of MMP-9, but not MMP-1, by human MN and alveolar macrophages.[14] Our experiments confirm this previous observation in MN and show for the first time that $1\alpha,25(OH)_2D_3$ also regulates *MMP-7* and *MMP-10* expression in MN under basal conditions and/or during *M. tuberculosis* infection. As previous studies have suggested that an interaction between T cells and MN/macrophages enhances MMP expression by both cell populations[11,22–25] we also investigated the effects of $1\alpha,25(OH)_2D_3$ on MMP regulation in a mixed population of adherent and non-adherent PBMC. Our observation that each MMP analysed had higher constitutive expression in PBMC than in MN led us to investigate the influence of $1\alpha,25(OH)_2D_3$ and *M. tuberculosis* on MMP secretion and activity in PBMC. We found that the only significant effect of $1\alpha,25(OH)_2D_3$ on basal MMP secretion by PBMC was a decrease in MMP-9, consistent with Lacraz *et al.*[14] More significantly, we found that there was a greater effect of $1\alpha,25(OH)_2D_3$ when cells were infected with *M. tuberculosis* (Table 1).

Consistent with previous observations in macrophages[4] we found that *M. tuberculosis* induced expression of *MMP-1* and *MMP-10* (in both PBMC and MN) and *MMP-7* (only in MN), but did not influence the expression of *MMP-9*. The cell-type specific effect on expression of *MMP-7* may reflect the fact that *MMP-7* is predominantly expressed in mononuclear phagocytes[35,36] or that interferon-γ produced from T lymphocytes in response to *M. tuberculosis* infection may be responsible for suppressing *MMP-7* expression in PBMC.[11] Significantly, $1\alpha,25(OH)_2D_3$ inhibited the expression of *MMP-7*, *MMP-9* and *MMP-10* in *M. tuberculosis*-infected PBMC, and this was reflected by a decrease in secretion of MMP-7 and MMP-9 and a decrease in MMP-9 activity. In contrast, the influence of $1\alpha,25(OH)_2D_3$ on the expression and secretion of TIMP was small. Taken together, these results suggest that $1\alpha,25(OH)_2D_3$ primarily influences MMP secretion and activity in *M. tuberculosis*-infected leucocytes at the transcriptional level.

We therefore investigated the effect of $1\alpha,25(OH)_2D_3$ on the secretion of known regulators of *MMP* gene expression, and found that $1\alpha,25(OH)_2D_3$ induced secretion of both PGE_2 (in MN and PBMC, irrespective of infection) and IL-10 (only in *M. tuberculosis*-infected PBMC). It has previously been reported that $1\alpha,25(OH)_2D_3$ augments the development of IL-10-secreting T helper type 2 cells in mice,[17] and this may explain our observation that induction of IL-10 by $1\alpha,25(OH)_2D_3$ was PBMC-specific. Both IL-10 and PGE_2 have been reported to suppress the expression and secretion of MMP[10,11,13] and it is possible that the effects of $1\alpha,25(OH)_2D_3$ on MMP expression that we report here are IL-10-mediated or PGE_2-mediated. Experiments to determine the effect of blocking the actions of IL-10 and PGE_2 on MMP expression in $1\alpha,25(OH)_2D_3$-treated cells are required to investigate this possibility.

Although excess MMP activity is implicated in the pathogenesis of TB, suppression of MMP activity also has the potential to impair host responses by inhibiting the recruitment of cells required to contain infection.[37] This dichotomy is illustrated in a study reporting that MMP-9 knockout mice infected with *M. tuberculosis* exhibit impaired macrophage recruitment and granuloma development, while administration of a broad-range MMP inhibitor to wild-type mice exposed to *M. tuberculosis* infection reduces haematogenous spread of infection.[38] In humans, administration of vitamin D to patients with pulmonary TB has been reported to reduce both cavitation[39] and time to sputum smear conversion[40] suggesting that vitamin D supplementation may afford a net clinical benefit associated with enhanced resolution of pulmonary cavitation. Clinical studies of the effect of vitamin D supplementation on MMP regulation in TB patients are required, and are a current focus of our work.

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Competing interests

The authors have no competing interests to disclose.

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