Primary research

The effects of \(1\alpha,25\)-dihydroxyvitamin D\(_3\) on matrix metalloproteinase and prostaglandin E\(_2\) production by cells of the rheumatoid lesion

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Statement of findings

The biologically active metabolite of vitamin D\(_3\), \(1\alpha,25\)-dihydroxyvitamin D\(_3\) \([1\alpha,25(OH)\_2D\_3]\), acts through vitamin D receptors, which were found in rheumatoid tissues in the present study. IL-1\(\beta\)-activated rheumatoid synovial fibroblasts and human articular chondrocytes were shown to respond differently to exposure to \(1\alpha,25(OH)\_2D\_3\), which has different effects on the regulatory pathways of specific matrix metalloproteinases and prostaglandin E\(_2\).

**Keywords:** \(1\alpha,25\)-dihydroxyvitamin D\(_3\), matrix metalloproteinase, prostaglandin E\(_2\), rheumatoid arthritis

Abstract

**Introduction:** \(1\alpha,25\)-dihydroxyvitamin D\(_3\) \([1\alpha,25(OH)\_2D\_3]\), the biologically active metabolite of vitamin D\(_3\), acts through an intracellular vitamin D receptor (VDR) and has several immunostimulatory effects. Animal studies have shown that production of some matrix metalloproteinases (MMPs) may be upregulated in rat chondrocytes by administration of \(1\alpha,25(OH)\_2D\_3\); and cell cultures have suggested that \(1\alpha,25(OH)\_2D\_3\) may affect chondrocytic function. Discoordinate regulation by vitamin D of MMP-1 and MMP-9 in human mononuclear phagocytes has also been reported. These data suggest that vitamin D may regulate MMP expression in tissues where VDRs are expressed.

Production of \(1\alpha,25(OH)\_2D\_3\) within synovial fluids of arthritic joints has been shown and VDRs have been found in rheumatoid synovial tissues and at sites of cartilage erosion. The physiological function of \(1\alpha,25(OH)\_2D\_3\) at these sites remains obscure. MMPs play a major role in cartilage breakdown in the rheumatoid joint and are produced locally by several cell types under strict control by regulatory factors. As \(1\alpha,25(OH)\_2D\_3\) modulates the production of specific MMPs and is produced within the rheumatoid joint, the present study investigates its effects on MMP and prostaglandin E\(_2\) \([\text{PGE}_2]\) production in two cell types known to express chondrolytic enzymes.

**Aims:** To investigate VDR expression in rheumatoid tissues and to examine the effects of \(1\alpha,25\)-dihydroxyvitamin D\(_3\) on cultured rheumatoid synovial fibroblasts (RSFs) and human articular chondrocytes (HACs) with respect to MMP and \(\text{PGE}_2\) production.

**Methods:** Rheumatoid synovial tissues were obtained from arthroplasty procedures on patients with late-stage rheumatoid arthritis; normal articular cartilage was obtained from lower limb amputations. Samples were embedded in paraffin, and examined for presence of VDRs by immunolocalisation using a biotinylated antibody and alkaline-phosphatase-conjugated avidin–biotin complex system. Cultured synovial fibroblasts and chondrocytes were treated with either \(1\alpha,25(OH)\_2D\_3\) or interleukin (IL)-1\(\beta\), or both. Conditioned medium was assayed for MMP and \(\text{PGE}_2\) by enzyme-linked immunosorbent assay (ELISA), and the results were normalised relative to control values.

**Results:** The rheumatoid synovial tissue specimens \((n = 18)\) immunostained for VDRs showed positive staining at...
In contrast, IL-1β by the simultaneous addition of 1α,25(OH)₂D₃ had a slight stimulatory effect on basal production of MMPs 1 and 3 by monolayer cultures of HACs, but stimulation of MMP-1 by IL-1β was not affected by the simultaneous addition of 1α,25(OH)₂D₃ whilst MMP-3 production was enhanced (Table 1). The production of PGE₂ by RSFs was unaffected by 1α,25(OH)₂D₃ addition, but when added concomitantly with IL-1β the expected IL-1β-stimulated increase was reduced to almost basal levels. In contrast, IL-1β stimulation of PGE₂ in HACs was not affected by the simultaneous addition of 1α,25(OH)₂D₃ (Table 2). Pretreatment of RSFs with 1α,25(OH)₂D₃ for 1 h made no significant difference to IL-1β-induced stimulation of PGE₂, but incubation for 16 h suppressed the expected increase in PGE₂ to control values. This effect was also noted when 1α,25(OH)₂D₃ was removed after the 16 h and the IL-1β added alone. Thus it appears that 1α,25(OH)₂D₃ does not interfere with the IL-1β receptor, but reduces the capacity of RSFs to elaborate PGE₂ after IL-1β induction.

**Discussion:** Cells within the rheumatoid lesion which expressed VDR were fibroblasts, macrophages, lymphocytes and endothelial cells. These cells are thought to be involved in the degradative processes associated with rheumatoid arthritis (RA), thus providing evidence of a functional role of 1α,25(OH)₂D₃ in RA. MMPs may play important roles in the chondrolytic processes of the rheumatoid lesion and are known to be produced by both fibroblasts and chondrocytes. The 1α,25(OH)₂D₃ had little effect on basal MMP production by RSFs, although more pronounced differences were noted when IL-1β-stimulated cells were treated with 1α,25(OH)₂D₃, with the RSF and HAC showing quite disparate responses. These opposite effects may be relevant to the processes of joint destruction, especially cartilage loss, as the ability of 1α,25(OH)₂D₃ to potentiate MMP-1 and MMP-3 expression by ‘activated’ chondrocytes might facilitate intrinsic cartilage chondrolysis in vivo. By contrast, the MMP-suppressive effects observed for 1α,25(OH)₂D₃ treatment of ‘activated’ synovial fibroblasts might reduce extrinsic chondrolysis and also matrix degradation within the synovial tissue. Prostaglandins have a role in the immune response and inflammatory processes associated with RA. The 1α,25(OH)₂D₃ had little effect on basal PGE₂ production by RSF, but the enhanced PGE₂ production observed following IL-1β stimulation of these cells was markedly suppressed by the concomitant addition of 1α,25(OH)₂D₃. As with MMP production, there are disparate effects of 1α,25(OH)₂D₃ on IL-1β stimulated PGE₂ production by the two cell types; 1α,25(OH)₂D₃ added concomitantly with IL-1β had no effect on PGE₂ production by HACs. In summary, the presence of VDRs in the rheumatoid lesion demonstrates that 1α,25(OH)₂D₃ may have a functional role in the joint disease process. 1α,25(OH)₂D₃ does not appear to directly affect MMP or PGE₂ production but does modulate cytokine-induced production.

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**Table 1**

|                 | Fibroblasts | Chondrocytes |
|-----------------|-------------|--------------|
| MMP-1 | MMP-3  | MMP-1 | MMP-3  |
| Control | 1 | 1 | 1 | 1 |
| + 1α,25D₃ | 1.03 ± 0.27 | 2.07 ± 0.35 | 1.38 ± 0.19 | 1.59 ± 0.22 |
| + IL-1 | 31.09 ± 4.97 | 31.28 ± 8.49 | 3.45 ± 0.49 | 9.05 ± 0.62 |
| + IL-1 + 1α,25D₃ | 15.55 ± 5.86 | 11.84 ± 2.82 | 3.71 ± 0.53 | 11.11 ± 0.31 |

Data given are normalized relative to control values and are expressed ± SEM for three cultures of each cell type.

**Table 2**

|                 | Fibroblasts | Chondrocytes |
|-----------------|-------------|--------------|
| MMP-1 | MMP-3  | MMP-1 | MMP-3  |
| Control | 1 | 1 | 1 | 1 |
| + 1α,25D₃ | 1.23 ± 0.16 | 1.35 ± 0.25 |
| + IL-1 | 7.07 ± 1.09 | 3.7 ± 1.05 |
| + IL-1 + 1α,25D₃ | 1.61 ± 0.7 | 4.23 ± 1.10 |

Data given are normalized relative to control values and are expressed ± SEM for three cultures of each cell type.
Full article

Introduction

The biologically active metabolite of vitamin D₃, 1,25-dihydroxyvitaminD₃ [1α,25(OH)₂D₃], acts through an intracellular receptor [vitamin D receptor (VDR)] and has a main role in the regulation of calcium and phosphorus metabolism [1]. It also has several immunomodulatory actions such as its effect on the differentiation and proliferation of T lymphocytes, and the regulation of immunoglobulin production by B lymphocytes [2–4]. 1α,25(OH)₂D₃ may affect chondrocytic function, such as proteoglycan and collagen synthesis [5]; and animal studies have shown that the production of some matrix metalloproteinases (MMPs), namely interstitial collagenase (MMP-1), stromelysin (MMP-3) and 72-kDa gelatinase (MMP-2), may be upregulated in rat chondrocytes by administration of the metabolite [6]. Discordant regulation by vitamin D of MMP-1 and MMP-9 in human mononuclear phagocytes has also been reported [7]. Together these data have suggested that vitamin D can regulate MMP expression in tissues or pathologies where receptors for the hormone are expressed.

The kidney is recognized as the primary source of 1α,25(OH)₂D₃, producing the metabolite via 1-hydroxylation of 25-hydroxyvitamin D₃ [1]. However, the local production of 1α,25(OH)₂D₃ within synovial fluids of arthritic joints, especially the macrophage component, has recently been indicated [8,9]; and receptors for vitamin D have also been demonstrated in rheumatoid synovial tissues and at sites of cartilage erosion [10]. Such studies have demonstrated a local source of 1α,25(OH)₂D₃ within the rheumatoid joint, but its regulation and physiological functions at this site remain obscure.

MMPs are reputed to play a major role in cartilage breakdown in the rheumatoid joint and are produced locally by several cell types, but especially by synovial fibroblasts and articular chondrocytes [11–16]. MMP production and release is microenvironmental in nature and is tightly regulated by several factors, including the proinflammatory cytokines tumour necrosis factor-α and interleukin (IL)-1β [17]. Because 1α,25(OH)₂D₃ has been shown to modulate the production of specific MMPs and is produced within the rheumatoid joint, the present study was designed to investigate the effects of 1α,25(OH)₂D₃ on MMP and prostaglandin E₂ (PGE₂) production by rheumatoid synovial fibroblasts (RSFs) and human articular chondrocytes (HACs), cell types known to express chondrolytic enzymes both in vitro and in vivo.

Methods

Tissue samples

Samples of rheumatoid synovial tissue, cartilage and cartilage–pannus junction were obtained from arthroplasty procedures performed on patients with classic late-stage rheumatoid arthritis. Normal articular cartilage samples were obtained from lower limb amputations. Samples were fixed in Carnoy’s fixative at 20°C for 2h, embedded in paraffin wax and 5µm sections cut. Tissue sections were dewaxed, rehydrated and examined for the presence of VDR.

Immunolocalization of vitamin D receptors

Tissue sections were treated with 2N HCl at 37°C for 30min, this being the antigen retrieval procedure recommended by the supplier of the primary antibody. Non-immune rabbit serum at 10% (vol : vol) in TRIS-buffered saline was applied to the sections for 20min at 20°C before incubation with the primary antibody. Rat monoclonal antibody to chick VDR (Biogenex, San Remo, USA), which is known to cross-react with human VDR, was applied to the sections for 2h at 20°C after dilution 1:40 in TRIS-buffered saline. After 3 x 10min washing in TRIS-buffered saline, biotinylated rabbit anti-rat immunoglobulin G (DAKO, Glostrup, Denmark) diluted 1:200 in TRIS-buffered saline was applied to the sections for 45min at 20°C. After further washing in TRIS-buffered saline, alkaline phosphatase-conjugated ABC (Avidin–biotin complex system; DAKO) was applied to the sections for 45min at 20°C, diluted as instructed by the supplier. After further washing the alkaline phosphatase was developed using new fuchsin substrate to give a red colour. Sections were lightly counterstained using Harris’s haematoxylin or toluidine blue. Non-immune rat immunoglobulin G was substituted for the primary antibody at similar concentrations on control tissue sections [10].

Cell cultures

Rheumatoid synovial tissue and human articular cartilage were enzymically digested to provide synovial fibroblast and chondrocyte cultures as previously described [18,19]. Cells were grown in Dulbecco’s Modified Eagle’s Medium + 10% (vol:vol) foetal calf serum, harvested and seeded into 12-well culture dishes (Nunc, Gibco, UK). Triplicate wells of confluent cell cultures in Dulbecco’s Modified Eagle’s Medium + 2% foetal calf serum were treated with 1α,25(OH)₂D₃ (10⁻⁸ mol/l), IL-1β (0.05 ng/ml), or IL-1β + 1α,25(OH)₂D₃ (0.05 ng/ml and 10⁻⁸ mol/l, respectively) and incubated for 48h at 37°C. The conditioned medium was collected and assayed for MMP-1, MMP-2, MMP-3 and MMP-9, and PGE₂ using enzyme-linked immunosorbant assay (ELISA) methodology. Cell numbers per well were counted at the end of each experiment after 70% ethanol fixation and toluidine blue staining.

Enzyme linked immunosorbent assays

ELISA methodology was used to determine protein levels of MMP-1 (collagenase 1), MMP-3 (stromelysin) and MMP-9 (gelatinase B) as previously described [20]. MMP-2 (Gelatinase A) was measured using ELISA kits pur-
chased from The Binding Site (Birmingham, UK); and 
PGE$_2$ was measured using an ELISA assay kit purchased 
from R & D Systems Europe, Ltd (Abingdon, UK).

All ELISA results were initially calculated in ng or pg 
protein/ml culture medium/10$^6$ cells per 48h. Three dif-
ferent cultures of both RSFs and HACs were examined, 
but the capacities of each cell type to produce the MMPs 
and PGE$_2$ varied between the individual cultures. There-
fore, the data from each culture was ‘normalized’ relative 
to control values, and the data sets from the three cultures 
of each cell type were subsequently pooled. This provided 
an evaluation that showed qualitative similarities for the 
RSFs and HACs, but demonstrated differences in 
1α,25(OH)$_2$D$_3$ responses by each of these two cell types.

Results

Demonstration of the vitamin D receptor in rheumatoid 
tissues in vivo

Specimens of rheumatoid synovial tissue ($n = 18$) immuno-
stained for VDR were shown to have variable distributions 
of the receptor. All specimens showed some positive stain-
ing, but this could be less than 5% or as much as 70% of 
the total cell population. Different cell types within the 
synovial specimens were shown to express the receptor, 
including macrophages, endothelial cells, lymphocytes 
and fibroblastic cells, but no regular pattern was observed. 
Cells with fibroblastic morphology immunostained for 
VDR are shown in Figure 1a. Chondrocytes within articular 
cartilage from rheumatoid joints also expressed the 
receptor in six out of 10 specimens (Fig. 1b), this being a 
much higher frequency compared with the one in 10 spec-
imens of normal articular cartilage from nonarthritic joints 
(data not shown). VDR-positive cells were also observed 
in association with some cartilage–pannus junctions, 
described here as the rheumatoid lesion (Fig. 1c).

Effects of 1α,25-dihydroxyvitamin D$_3$ on matrix 
metalloproteinase production by rheumatoid synovial 
fibroblasts

1α,25(OH)$_2$D$_3$ alone had no effect on basal MMP produc-
tion by RSFs in monolayer culture, but the simultaneous 
addition of 1α,25(OH)$_2$D$_3$ with IL-1β reduced the 
expected stimulation of MMP-1, MMP-3 and MMP-9 by 
up to 50% (Fig. 2: $P = 0.096, 0.009$ and $0.01$, for IL-1β versus 
IL-1β + 1α,25(OH)$_2$D$_3$ for MMP-1, MMP-3 and MMP-9, 
respectively, by Student’s $t$-test). MMP-2 production was 
not affected by either IL-1β or IL-1β + 1α,25(OH)$_2$D$_3$ (data 
not shown), an observation that is in accord with the consti-
tutive nature of MMP-2 expression [21].

Effects of 1α,25-dihydroxyvitamin D$_3$ on matrix 
metalloproteinase production by human articular 
chondrocytes

In contrast to the data for RSFs, 1α,25(OH)$_2$D$_3$ had a slight 
stimulatory effect on basal production of MMP-1 and

\[\text{Figure 1}\]

Immunolocalization of the vitamin D receptor (VDR) in rheumatoid 
tissues. (a) Immunolocalization of VDR in rheumatoid synovium. Note 
positive red immunostaining of fibroblastic cells. (Counterstain Harris’s 
haematoxylin; bar = 25 μm.) (b) Demonstration of VDR in cartilage 
from a rheumatoid joint. Note both positive and negative chondrocytes. 
(Counterstain Harris’s haematoxylin; bar = 20 μm.) (c) VDR immuno-
localization at the cartilage–pannus junction; cells within both pannus 
tissue and cartilage can be seen to be expressing the receptor. 
(Counterstain toluidine blue; bar = 25 μm.)
MMP-3 by monolayer cultures of HAC (Fig. 3: \( P = 0.098 \) and 0.002, for control versus 1\( \alpha \),25(OH)\(_2\)D\(_3\), for MMP-1 and MMP-3, respectively, by Student’s \( t \)-test). When stimulated with IL-1\( \beta \) MMP-1 and MMP-3 production was increased, and although simultaneous addition of 1\( \alpha \),25(OH)\(_2\)D\(_3\) had no effect on the stimulation of the MMP-1 enzyme, MMP-3 production was further enhanced (Fig 3b: \( P = 0.008 \), by Students \( t \)-test). MMP-9 and MMP-2 were not produced in measurable quantities by these HAC cultures, either with or without IL-1\( \beta \) stimulation. 

Effects of 1\( \alpha \),25-dihydroxyvitamin D\(_3\) on prostaglandin E\(_2\) production by rheumatoid synovial fibroblasts and human articular chondrocytes

PGE\(_2\) production by RSFs was unaffected by the addition of 1\( \alpha \),25(OH)\(_2\)D\(_3\) alone. Treatment of RSFs with IL-1\( \beta \) upregulated the production of PGE\(_2\), but the addition of 1\( \alpha \),25(OH)\(_2\)D\(_3\) together with IL-1\( \beta \) reduced the expected stimulation of PGE\(_2\) almost to control values (Fig. 4a: http://arthritis-research.com/14oct99/ar0101p01).
$P = 0.014$, for IL-1$\beta$ versus IL-1$\beta$ + 1$\alpha$,25(OH)$_2$D$_3$, by Student’s $t$-test).

Treatment of HACs with IL-1$\beta$ also increased the production of PGE$_2$, but in contrast to the effects noted for RSFs this IL-1-stimulation of PGE$_2$ was not affected by the concomitant addition of 1$\alpha$,25(OH)$_2$D$_3$ (Fig. 4b).

To examine the possibility that 1$\alpha$,25(OH)$_2$D$_3$ might obscure or interact with the IL-1$\beta$ receptor of RSFs the latter were pretreated with 1$\alpha$,25(OH)$_2$D$_3$ before incubation with IL-1$\beta$. Figure 4c shows that a 1-h preincubation with 1$\alpha$,25(OH)$_2$D$_3$ followed by IL-1$\beta$ was not significantly different from the two factors added together, but preincubation with 1$\alpha$,25(OH)$_2$D$_3$ for 16 h suppressed the expected increase in PGE$_2$ production to control values. This effect was noted even when the 1$\alpha$,25(OH)$_2$D$_3$ was removed after the 16 h and IL-1$\beta$ then added alone (Fig. 4c, data column F). Thus, rather than directly interfering with the IL-1$\beta$ receptor, it appears that 1$\alpha$,25(OH)$_2$D$_3$ reduces the capacity of the RSFs to elaborate PGE$_2$ (and probably the MMPs shown in Fig. 2) after IL-1$\beta$ induction.

**Discussion**

The cell types within the rheumatoid lesion which were observed to express VDR included chondrocytes, fibroblasts, macrophages, lymphocytes and endothelial cells. These cells are all purported to be involved either directly or indirectly in the degradative processes associated with rheumatoid arthritis, possibly via their MMP and prostanoid production, or via the production of mediators responsible for inflammation and induction of proteinase expression by other cell types. Thus, the demonstration of VDR within the rheumatoid lesion provides support for a functional role of 1$\alpha$,25(OH)$_2$D$_3$ in rheumatoid arthritis.

MMPs are considered to play important roles in the chondrolytic processes of the rheumatoid lesion [14,15,17]. These enzymes are known to be produced by both fibroblasts and chondrocytes, but little has been reported in the literature regarding a relationship between 1$\alpha$,25(OH)$_2$D$_3$ and MMP production or its regulation, and most of the data to date have been obtained from animal studies or immortalized cell lines [5–7]. 1$\alpha$,25(OH)$_2$D$_3$ had little effect on basal MMP production by RSFs and marginally increased the basal production of MMP-1 and MMP-3 by chondrocytes. More pronounced differences were noted when IL-1$\beta$-stimulated or activated cells were treated with 1$\alpha$,25(OH)$_2$D$_3$, the RSFs and HACs showing quite disparate responses. These opposite effects may be of relevance to the processes of joint destruction, especially cartilage loss, because the ability of 1$\alpha$,25(OH)$_2$D$_3$ to potentiate MMP-1 and MMP-3 expression by ‘activated’ chondrocytes might facilitate intrinsic cartilage chondrolysis in vivo. By contrast, the MMP-suppressive effects observed for 1$\alpha$,25(OH)$_2$D$_3$ treatment of ‘activated’ synovial fibroblasts with IL-1$\beta$ + 1$\alpha$,25(OH)$_2$D$_3$, by Student’s t-test).
lasts might reduce extrinsic chondrolysis and also matrix degradation within the synovial tissue. We recognize that the present study is somewhat restricted to the 1α,25(OH)2D3 effects on MMP-1 and MMP-3 production. Although these are prominent and well characterized MMPs, there are many other enzymes in this family, together with plasminogen activators and other proteinases, which have not been examined. From the disparate effects of 1α,25(OH)2D3 on the RSFs and HACs it would seem that further studies on the 1α,25(OH)2D3-modified proteinase phenotypes of these cells are warranted.

Prostaglandins are primary mediators of inflammation and have important roles in the immune response and the inflammatory processes associated with rheumatoid arthritis, and PGE2 has been implicated in the potentiation of MMP production by some cell cultures [22,23]. 1α,25(OH)2D3 had little effect on basal PGE2 production by RSFs, but the enhanced PGE2 production observed following IL-1β stimulation of these cells was markedly suppressed by the concomitant addition of 1α,25(OH)2D3. By contrast, the increased PGE2 production observed for IL-1β-treated HACs was unaffected by the simultaneous addition of 1α,25(OH)2D3. Thus, as with MMP production, 1α,25(OH)2D3 has disparate effects on IL-1β-stimulated PGE2 production by these two cell types. Different responses by RSFs and HACs to the same ligand have been noted before; for example, IL-1β treatment was shown to stimulate glycosaminoglycan synthesis by RSFs, but inhibited its production by chondrocytes [24].

In summary, the immunolocalization of VDR in the rheumatoid lesion has demonstrated that the metabolite 1α,25(OH)2D3 might have a functional role in the degradative and inflammatory processes of joint disease. Whereas 1α,25(OH)2D3 does not appear directly to affect the MMP or prostanooid production by unstimulated RSFs or HACs in vitro, it was shown to modulate the cytokine-induced MMP and PGE2 production by these two cell cultures. The recognized immunomodulatory properties of 1α,25(OH)2D3 could well be important in rheumatoid tissues, in which the inflammatory response is a characteristic feature. The transient, local manifestations of cartilage and matrix-degrading activity [25] could be modified by 1α,25(OH)2D3 if the cells present express VDR and the metabolite is produced locally. This study has demonstrated that most rheumatoid synovial specimens were positive for VDR, and 1α,25(OH)2D3 has disparate effects on IL-1β-stimulated pro- and immunomodulatory properties [1,7].

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References

1. Norman AW, Roth J, Orci L: The vitamin D endocrine system: steroid metabolism, hormone receptors and biological response (calcium binding proteins). Endocr Rev 1982, 3:331–336.
2. Suda T: The role of 1α,25-dihydroxyvitamin D3 in myeloid cell differ-entiation. Proc Soc Exp Biol Med 1989, 191:214–220.
3. Lemire JM: Immunomodulatory actions of 1,25-dihydroxyvitamin D3. J Ster Biochem Mol Biol 1995, 53:599–602.
4. Lemire JM: Immunomodulatory role of 1,25-dihydroxyvitamin D3. J Cell Biochem 1992, 49:26–31.
5. Gerstenfeld LC, Kelly CM, von Deck M, Lian JB: Effect of 1,25-dihydroxyvitamin D3 on induction of chondrocyte maturation in culture: extracellular matrix gene expression and morphology. Endocrinology 1990, 126:1599–1609.
6. Dean DD, Schwartz Z, Schmitz J, et al: Vitamin D regulation of met-alloproteinase activity in matrix vesicles. Conn Tiss Res 1996, 35:385–390.
7. Lacraz S, Dayer J-M, Welgus HG: 1,25-dihydroxyvitamin D3 dissociates production of interstitial collagenase and 92 kDa gelatinase in human mononuclear phagocytes. J Biol Chem 1994, 269:6485–6490.
8. Mawer EB, Hayes ME, Still PE, et al: Evidence for non-renal synthesis of 1,25-dihydroxyvitamin D in patients with inflammatory arthritis. J Bone Miner Res 1991, 6:733–739.
9. Hayes ME, Denton J, Freemont AJ, Mawer EB: Synthesis of the active metabolite of vitamin D, 1,25(OH)2D3 by synovial fluid macrophages in arthritic diseases. Ann Rheum Dis 1989, 48: 723–729.
10. Tetlow LC, Smith SJ, Mawer EB, Woolley DE: Vitamin D receptors in the rheumatoid lesion: expression by chondrocytes, macrophages and synoviocytes. Ann Rheum Dis 1999, 58:118–121.
11. Woolley DE, Harris Ed Jr, Mainardi CL, Brinckerhoff CE: Collagenase immunolocalisation in cultures of rheumatoid synovial cells. Science 1978, 200:773–775.
12. Unemori EN, Hibbs MS, Amento EP: Constitutive expression of a 92-kDa gelatinase by rheumatoid synovial fibroblasts by inflam-matory cytokines. J Clin Invest 1991, 88:1656–1662.
13. Tetlow LG, Lees M, Woolley DE: Comparative studies of collage-nase and stromelysin 1 expression by rheumatoid synoviocytes in vitro. Virchows Arch B: Cell Pathol 1995, 425:569–576.
14. Okada Y, Taakeuchi N, Tomita K, Nakashima I, Nagase H: Immunolocalisation of matrix metalloproteinase-3 (stromelysin) in rheuma-toid synovioblasts (RBS cells): correlation with rheumatoid arthritis. Ann Rheum Dis 1989, 48:645–653.
15. Gravelllese EM, Darling JM, Ladd AL, Katz JN, Glimcher LH: In situ hybridisation studies of stromelysin and collagenase mRNA expression in rheumatoid synovium. Arthritis Rheum 1991, 34: 1076–1084.
16. Tetlow LC, Woolley DE: Comparative immunolocalisation studies of collagenase 1 and collagenase 3 production in the rheumatoid lesion and by human chondrocytes and synoviocytes in vitro. Br J Rheumatol 1998, 37:64–70.
17. Birkedal-Hansen H: Matrix metalloproteinases: a review. Crit Rev Oral Biol 1993, 4:197–250.
18. Dayer JM, Krane SSM, Russell RGG, Robinson DR: Production of collagenase and prostaglandins by isolated adherent rheumatoid synovial cells. Proc Natl Acad Sci U S A 1976, 73:945–949.
19. Meats JE, McGuire MK, Russell RGG: Human synovium releases a factor which stimulates chondrocyte production of PGE and plasminogen activator. Nature 1980, 286:891–892.
20. Tetlow LC, Harper N, Dunningham Tet al: Effects of induced mast cell activation on prostaglandin E and metalloproteinase produc-tion by rheumatoid synovial tissue in vitro. Ann Rheum Dis 1998, 57:25–32.
21. Okada Y, Morodomi T, Enghild JJ, et al: Matrix metalloproteinase 2 from human rheumatoid synovial fibroblasts: purification and acti-vation of the precursor and enzymatic properties. Eur J Biochem 1990, 194:721–730.
22. Goodwin JS: Are prostaglandins proinflammatory, antiinflammatory, both or neither? J Rheumatol 1991, 18 (suppl 28):26–29.

23. Dayer J-M, Goldring SR, Robinson DR, Krane SM: Cell–cell interactions and collagenase production. In: Collagenase in Normal and Pathological Connective Tissues. Edited by Woolley DE, Evanson JM. Chichester, UK: John Wiley & Sons; 1980:83–104.

24. Yaron I, Meyer FA, Dayer J-M, Bleiberg I, Yaron M: Some recombinant human cytokines stimulate glycosaminoglycan synthesis in human synovial fibroblast cultures and inhibit it in human articular cartilage cultures. Arthritis Rheum 1989, 32:173–176.

25. Woolley DE, Tetlow LC: Observations on the microenvironmental nature of cartilage degradation in rheumatoid arthritis. Ann Rheum Dis 1997, 56:151–161.

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