Inhibition by prostaglandin E₁ and E₂ of 1,25-dihydroxyvitamin D₃ synthesis by synovial fluid macrophages from arthritic joints

M E Hayes, A Rai, R G Cooper, D Bayley, A J Freemont, E B Mawer

Abstract

Previous work has shown that renal metabolism of 25-dihydroxyvitamin D₃ (25(OH)D₃) to the active metabolite, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) is stimulated by prostaglandin E₂ and inhibited by acetylsalicylate (aspirin). As prostaglandins are primary inflammatory mediators and synovial fluid macrophages are known to synthesise 1,25(OH)₂D₃ in vitro, the effects of prostaglandin E₁, prostaglandin E₂, and aspirin on the metabolism of 25(OH)D₃ by cells cultured from synovial fluid of patients with inflammatory arthritis were investigated. Most cultures contained non-proliferating macrophages which formed 1,25(OH)₂D₃; however, two of 13 cultures contained colonies of rapidly proliferating fibroblast-like cells which formed 24,25(OH)₂D₃ (24,25(OH)₂D₃).

Prostaglandin E₁ and prostaglandin E₂ (0.01–10 μmol/l) induced marked inhibition of 1,25(OH)₂D₃ synthesis (up to 94%) in a dose dependent manner after preincubations of 24 hours but not over straightforward six hour incubations. Exposure of macrophages to aspirin (1 μmol/l–1 mmol/l) for 24 hours did not affect 1,25(OH)₂D₃ synthesis unless the cells had been pretreated with lipopolysaccharides, in which instance 1 mM aspirin increased 1,25(OH)₂D₃ synthesis. Lipopolysaccharide is a macrophage activating factor which stimulates macrophages to form 1,25(OH)₂D₃, and it also induces prostaglandin synthesis which would be inhibited by aspirin. Taken together these results suggest that prostaglandin E₁ and prostaglandin E₂ synthesised by macrophages may act in an autocrine manner to attenuate the ability of macrophage activating factors, such as lipopolysaccharide, to stimulate 1,25(OH)₂D₃ synthesis. Prostaglandins synthesised by other inflammatory cells may also inhibit 1,25(OH)₂D₃ synthesis in a paracrine manner. In contrast, prostaglandin E₂ and aspirin had limited effects on fibroblast 24,25(OH)₂D₃ synthesis.

This study shows that the effects of prostaglandin E₁, prostaglandin E₂, and aspirin in macrophages contrast with those previously reported for the renal 25(OH)D₃-1α-hydroxylase, where prostaglandin E₂ stimulated and aspirin inhibited enzyme activity. These results further emphasise that synthesis of 1,25(OH)₂D₃ in non-renal sites is independently regulated, which is consistent with it having an immunological role at a local level rather than playing a part in systemic calcium homeostasis.

Vitamin D₃, which is formed in the skin or provided by the diet, is rapidly hydroxylated to 25-dihydroxyvitamin D₃ (25(OH)D₃) in the liver and released into the blood where it is carried by a specific vitamin D binding protein. 25(OH)D₃ has limited biological activity but instead provides a serum pool available for secondary hydroxylation to 24,25-dihydroxyvitamin D₃ (24,25(OH)₂D₃) and 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) the most biologically active metabolite with respect to calcium metabolism and immune function.^{1–4} The two major cell types responsible for the synthesis of 1,25(OH)₂D₃ are endothelial cells of the proximal renal tubule and activated tissue macrophages.

Control of renal 25(OH)D₃ metabolism is primarily linked with regulation of intestinal calcium absorption and homeostasis of blood calcium levels. The predominant renal metabolite formed during normocalcaemia is 24,25(OH)₂D₃, which does not itself appear to modulate blood calcium. However, when blood calcium levels decrease parathyroid hormone is secreted to promote synthesis of 1,25(OH)₂D₃, which stimulates intestinal calcium absorption.^{1,2}

Studies on the extrarenal metabolism of 25(OH)D₃ by cultured macrophages obtained from patients with sarcoidosis,⁵ tuberculosis,⁶ various forms of inflammatory arthritis,^{7,8} or following peritonitis⁹ have shown that 1,25(OH)₂D₃ is always the primary product. Monocyte macrophages maintained in short term culture may, however, in addition to fibroblasts cultured from skin or synovial fluid, form 24,25(OH)₂D₃.^{8,10} Synthesis of 1,25(OH)₂D₃ by macrophages is stimulated by macrophage activating factors, including γ interferon,¹¹ bacterial lipopolysaccharides,¹² and tumour necrosis factor α,¹³ and is inhibited by hydrocortisone,¹⁴ whereas 24,25(OH)₂D₃ synthesis by fibroblasts is stimulated by 1,25(OH)₂D₃.⁸ In a range of diseases, including arthritis, it has been shown that extrarenal synthesis of 1,25(OH)₂D₃ is substrate dependent and is poorly regulated, in contrast with renal synthesis.^{14–16}

It has also been shown that the locally synthesised inflammatory mediator prostaglandin E₂ stimulates renal 1,25(OH)₂D₃ synthesis and that aspirin, a prostaglandin inhibitor, reduces 1,25(OH)₂D₃ synthesis in vitro.¹⁷ In many forms of arthritis, locally synthesised prostaglandins are believed to be primary mediators of inflammation and associated tissue damage, including periarticular bone loss, whereas aspirin is commonly used as

Manchester University
Bone Disease Research
Centre and University
Departments of
Medicine and
Rheumatology,
The Royal Infirmary,
Manchester M13 9WL,
United Kingdom
M E Hayes
A Rai
D Bayley
A J Freemont
E B Mawer
The Rheumatic
Disease Centre,
Hope Hospital,
Salford M6 8HD,
United Kingdom
R G Cooper

Correspondence to:
Dr M E Hayes,
University Department
of Medicine,
The Royal Infirmary,
Manchester, Oxford Road,
Manchester M13 9WL,
United Kingdom.

Accepted for publication
30 August 1991

an anti-inflammatory drug.¹⁸ As a result of the effects of prostaglandin E₂ and aspirin on renal 25(OH)D₃-1 α -hydroxylase, the primary aim of this study was to assess the effects of these factors on the enzyme expressed by arthritic joint macrophages. It is difficult to isolate macrophages from arthritic synovium; however, it is relatively straightforward to isolate cells from synovial fluid which actively synthesise 1,25(OH)₂D₃ in culture. We therefore cultured macrophages from synovial fluid and investigated the effects of prostaglandin E₁, prostaglandin E₂, and aspirin on 1,25(OH)₂D₃ synthesis. We also compared the effects of these substances on 24,25(OH)₂D₃ synthesis by synovial fluid fibroblasts.

Patients and methods

Samples of synovial fluid were obtained from 13 patients with inflammatory arthritic knee joint effusions. Most of the patients were assigned to well recognised diagnostic groups on the basis of clinical criteria, the total and differential white cell counts on synovial fluid, and appropriate immunological, radiological, and crystallographic investigations. Eight of the patients had rheumatoid arthritis and two had reactive arthritis. The other three patients had arthropathies that could not be assigned to a specific diagnostic group and were classified as non-specific inflammatory arthritis.

Cells were harvested from synovial fluid (10–20 ml) by centrifugation at 2000 *g* for 15 minutes followed by resuspension in 10–20 ml of RPMI 1640 culture medium containing 10% newborn calf serum, 2 mmol/l glutamine, 50 IU/ml penicillin, and 50 μ g/ml streptomycin (Gibco). Cells were then plated as 0.5 ml aliquots into six or 24 well culture dishes (Imperial Laboratories) containing 2 or 10 ml of the RPMI 1640 culture medium respectively. Following one to two days culture in an atmosphere of 95% air, 5% carbon dioxide at 37°C the non-adherent polymorphs, lymphocytes, and monocytes were decanted, leaving adherent macrophages in most samples, mixed macrophages and fibroblasts in one culture, and fibroblasts in another. The macrophages were cultured for three to 42 days and the fibroblasts for 24 to 26 days in fresh RPMI 1640 medium which was routinely changed twice a week (see figure legends for details). Some of the macrophages were also cultured in medium containing lipopolysaccharides (20 μ g/ml) added to maintain the cells in an activated state and to increase the rate of 1,25(OH)₂D₃ synthesis.⁸ The experiments initially examined the ability of macrophages to synthesise 1,25(OH)₂D₃ after six and 24 hours exposure to prostaglandin E₁ or prostaglandin E₂, whereas other experiments tested the effects of aspirin on the ability of macrophages to synthesise 1,25(OH)₂D₃ and fibroblasts to form 24,25(OH)₂D₃.

The assays of 25(OH)D₃ metabolism were carried out *in situ* in 2 ml of serum free RPMI 1640 incubation medium containing 1.5 mg/ml of bovine serum albumin (Immuno Diagnostika), 50 000 dpm 25(OH)D₃ labelled with tritium (6.5 TBq/mmol, Amersham Inter-

national), 0.125 nmol 25(OH)D₃ (25 ng/ml, Roussel UCLAF) for six or 24 hours in an atmosphere of 95% air, 5% carbon dioxide at 37°C. Incubations were carried out in triplicate for each concentration of test substance and were terminated by mixing the cells and medium with 3 ml of chloroform and 2 ml of methanol. The extracts were centrifuged at 500 *g* for 20 minutes at 4°C and the lower chloroform layer containing vitamin D₃ metabolites was removed, dried under a stream of nitrogen gas, resuspended in 1 ml of ethanol and stored at –20°C. Each chloroform extract was analysed on a manual injection normal phase high performance liquid chromatography system, developed with a mobile phase of *n*-hexane-propan-2-ol-methanol (110: 6: 4 v/v) at 2 ml/min using a Model 6000A high performance liquid chromatography solvent delivery pump (Waters Associates) and a Zorbax-Sil column (25 cm $\times$ 4.6 mm, Du Pont). For each analysis eluent fractions were collected using a Model 202 fraction collector (Gilson) and the radioactivity associated with the substrate, 24,25(OH)₂D₃, or 1,25(OH)₂D₃ was estimated using a 1217 Rackbeta liquid scintillation counter (LKB Wallac). Standard 25(OH)D₃, 24,25(OH)₂D₃, and 1,25(OH)₂D₃ and their tritiated forms were also chromatographed and the retention times of these markers determined by ultraviolet absorption at 265 nm using a Model 440 absorbance detector (Waters Associates) with an Ar 25 linear chart recorder (Pye Unicam), or by liquid scintillation counting.

For each experiment parallel cultures of cells were trypsinised and counted in triplicate using a haemocytometer and the values used to normalise 25(OH)D₃-1 α -hydroxylase activity for cell numbers. Cytocentrifuge preparations of trypsinised cells and cells fixed *in situ* with methanol were stained with Giemsa. These preparations were used to confirm that the cells were macrophages and to check for the presence of fibroblast-like cell colonies.

Synthesis of 1,25(OH)₂D₃ and 24,25(OH)₂D₃ is expressed as pmol per hour per 10⁶ cells (means (SEM) of triplicate determinations). Statistical analysis was carried out using Student's *t* test with significance expressed as NS (not significant), *p*<0.05 (*), or *p*<0.01 (**) compared with controls.

Results

Most cultures were characterised by the presence of non-proliferating cells which initially had the morphology of mononucleated monocytes and immature macrophages, but after several days in culture had the appearance of larger, more mature macrophages, some of which were multinucleated (more than three nuclei). Addition of lipopolysaccharide to the macrophage cultures induced a more elongated morphology, but it did not appear to alter the cell number or the proportion of multinucleated cells present. Cultures from one sample contained rapidly proliferating colonies of fibroblast-like cells in addition to macrophages, whereas another was predominantly fibroblasts. All the macrophage cultures formed 1,25(OH)₂D₃ whereas the fibroblasts formed 24,25(OH)₂D₃.

Figure 1 Results of high performance liquid chromatographic analyses (HPLC) of metabolism of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) labelled with tritium by macrophages in a standard incubation medium for six hours following culture for 14 days in either autologous synovial fluid (●—●), normal RPMI 1640 culture medium (●---●), or medium containing 20 µg/ml lipopolysaccharide for the last five days (●—■), following isolation from the synovial fluid of a patient with non-specific inflammatory arthritis. Each point represents the radioactivity in eluent fractions collected following separation in a normal phase high performance liquid chromatography system developed with n-hexane, isopropanol, methanol (110:4:4 v/v) at a flow of 2 ml/min. The arrows show the retention times of standard 25-hydroxyvitamin D₃ 25(OH)D₃, 24,25-dihydroxyvitamin D₃ 24,25(OH)₂D₃, and 1,25(OH)₂D₃, which were determined by ultraviolet absorbance at 265 nm.

Figure 2 Effects of prostaglandin E₁ (●—●) and prostaglandin E₂ (●---●) after 24 hours on 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) synthesis by macrophages isolated from the synovial fluid of patients with (a) non-specific inflammatory arthritis, (b) rheumatoid arthritis, or (c) reactive arthritis. Assays of 25-hydroxyvitamin D₃ 1-hydroxylase activity were carried out for six hours using 0.15–0.35 × 10⁶ macrophages per incubation, which had been exposed to 20 µg/ml lipopolysaccharide for 5, 5, 4, 5, or 14 days and cultured for a total of 15, 20, 6, 13, or 15 days respectively in descending order. Values are the mean (SEM) of three determinations (*p<0.05; **p<0.01).

Figure 1 shows high performance liquid chromatography results for metabolism of 25(OH)D₃ labelled with tritium by macrophages cultured from the synovial fluid of a patient with non-specific inflammatory arthritis. The cells were incubated in medium for six hours following 14 days of culture in autologous synovial fluid, in normal RPMI 1640 culture medium, or in medium which contained 20 µg/ml lipopolysaccharide for the last five days. The macrophages cultured in autologous synovial fluid actively synthesised 1,25(OH)₂D₃, those cultured in medium containing lipopolysaccharides formed most 1,25(OH)₂D₃, whereas those cultured in the absence of lipopolysaccharides synthesised little 1,25(OH)₂D₃. Although lipopolysaccharides typically increase 25(OH)D₃-1α-hydroxylase activity, macrophages from some samples cultured in the absence of lipopolysaccharides synthesised considerable amounts of 1,25(OH)₂D₃, indicating that the cells were probably activated (compare figs 2 and 3).

Figure 2 shows that prostaglandin E₁ and prostaglandin E₂ inhibited 1,25(OH)₂D₃ synthesis in lipopolysaccharide activated macrophages in a dose dependent manner after a preincubation of 24 hours. The macrophages

Figure 3 Effects of prostaglandin E₂ on 1,25-dihydroxyvitamin D₃ 1,25(OH)₂D₃ synthesis by macrophages isolated from the synovial fluid of patients with (△, ▲) non-specific inflammatory arthritis, (○, ●) rheumatoid arthritis, or (□, ■) reactive arthritis. Assays of 25(OH)D₃-1-hydroxylase activity were carried out for two to six hours in the absence (open symbols) or presence of 1 µM prostaglandin E₂ (closed symbols) using 0.07–0.15 × 10⁶ macrophages per incubation, which had not been exposed to lipopolysaccharide but had been cultured for a total of three, 20, and 42 days respectively in ascending order. Values are the mean (SEM) of three determinations.

exposed to prostaglandin E₁ were from one patient with reactive arthritis and one with a non-specific inflammatory arthritis, whereas those exposed to prostaglandin E₂ were all from patients with active rheumatoid arthritis. Prostaglandin E₂ also inhibited 1,25(OH)₂D₃ synthesis in cells from two patients with rheumatoid arthritis that had not been exposed to lipopolysaccharides in the latter experiments, synthesis was reduced from 1.81 (0.05) and 2.44 (0.19) to 0.7 (0.15) ($p < 0.05$) and 0.95 (0.28) ($p < 0.01$) pmol 1,25(OH)₂D₃ per hour per 10⁶ cells respectively. Prostaglandin E₂, however, did not affect 1,25(OH)₂D₃ synthesis in straightforward six hour incubations (fig 3). Figure 3 also shows the almost linear synthesis of 1,25(OH)₂D₃ over the six hour incubation period used. In a further study using fibroblasts in which 25-hydroxyvitamin D₃ 24-hydroxylase had been induced by treatment with 10 mM 1,25(OH)₂D₃ for three days, 1 μ M prostaglandin E₂ (24 hours) did not inhibit 24,25(OH)₂D₃ synthesis (64.4 (1.45) pmol per hour per 10⁶ cells) compared with controls (59.5 (1.99) pmol per hour per 10⁶ cells).

Figure 4 shows that exposure to synovial fluid macrophages from patients with rheumatoid arthritis to aspirin for 24 hours did not affect 1,25(OH)₂D₃ synthesis, whereas 1 mM aspirin significantly increased 1,25(OH)₂D₃ synthesis ($p < 0.01$) by cells that had been exposed to lipopolysaccharides. In another experiment using mixed cultures of lipopolysaccharides activated macrophages and fibroblasts (0.2×10^6 cells per incubation), 1 mM aspirin also increased 1,25(OH)₂D₃ synthesis from 0.41

Figure 4 Effects of aspirin on 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) synthesis by macrophages isolated from the synovial fluid of three patients with rheumatoid arthritis. Assays of 25(OH)D₃-1 α -hydroxylase activity were carried out for 24 hours using $0.15\text{--}0.45 \times 10^6$ macrophages per incubation following culture in the presence of 20 μ M lipopolysaccharide for 14 days (■- -■) or in its absence (●—●). The cells had been cultured for a total of 14, seven, and six days respectively in descending order and the values are the mean (SEM) of three determinations (** $p < 0.01$).

(0.02) to 0.85 (0.08) pmol per hour per incubation ($p < 0.01$) and reduced 24,25(OH)₂D₃ synthesis from 0.72 (0.06) to 0.55 (0.02) pmol per hour per incubation (NS). Neither aspirin (1 μ mol/l–1 mmol/l) nor lipopolysaccharide, however, altered the rate of 24,25(OH)₂D₃ synthesis by fibroblasts from a patient with rheumatoid arthritis where control cells synthesised 5.0 (0.6) pmol per hour per 10⁶ cells.

The ability of the cells to metabolise 25(OH)D₃ with respect to the type of arthritis, clinical treatment, or number of days in culture was not examined due to the small number of samples.

Discussion

This study confirms observations made in our previous studies that macrophages cultured from the synovial fluid of patients with various forms of arthritis can synthesise 1,25(OH)₂D₃, whereas fibroblast-like cells synthesise 24,25(OH)₂D₃.^{7,8} Our previous studies have also shown that lipopolysaccharides increase and maintain high rates of 1,25(OH)₂D₃ synthesis.⁸ In this study a number of the samples expressed high rates of 25(OH)D₃-1 α -hydroxylase activity (>8 pmol per hour 10⁶ cells) without previous exposure to lipopolysaccharides, indicating that the cells may already have been activated before culture. It was also shown that macrophages cultured in autologous synovial fluid are capable of synthesising 1,25(OH)₂D₃.

These studies have clearly shown that 1 μ mol/l concentrations of prostaglandin E₁ and prostaglandin E₂ inhibit 1,25(OH)₂D₃ synthesis by over 60% in untreated and in lipopolysaccharide activated macrophages in vitro after 24 hour preincubations, but not over straightforward six hour incubations. In contrast, 1 mM aspirin, an inhibitor of prostaglandin synthesis, significantly increased 1,25(OH)₂D₃ production by lipopolysaccharide activated macrophages but not by untreated cells. Lipopolysaccharides are known inducers of prostaglandin E₂ synthesis in monocyte macrophages,^{18,19} thus it is likely that lipopolysaccharide induced prostaglandin E₂ synthesis in the macrophages may have attenuated the ability of lipopolysaccharides to stimulate 25(OH)D₃-1 α -hydroxylase activity in an auto-crine manner. The effects of aspirin in lipopolysaccharide activated macrophages may therefore have been mediated by its ability to inhibit prostaglandin E₂ synthesis. In the studies described by Fidler *et al* the concentrations of prostaglandin E₂ achieved after 18 hour incubations of 10⁵ human monocytes exposed to 1 μ g/ml lipopolysaccharide were 30–40 nmol/l.¹⁹ Such concentrations were within the range that inhibited 1,25(OH)₂D₃ synthesis in this study.

The effects of prostaglandin E₁, prostaglandin E₂, and aspirin in macrophages are in complete contrast with those previously reported for the renal 25(OH)D₃-1 α -hydroxylase, where prostaglandin E₂ stimulated and aspirin inhibited enzyme activity.¹⁷ This further emphasises the different functions (calcium homeostasis *v* immunoregulation) and regulatory

factors (parathyroid hormone *v* cytokines) for the two 25(OH)D₃-1 α -hydroxylases. These studies also indicate that prostaglandins and aspirin have limited effects on 25(OH)D₃-24-hydroxylase activity in cultured synovial fluid fibroblast-like cells.

There is now growing evidence that 1,25(OH)₂D₃ may have important immunoregulatory functions in a number of clinical disorders.²⁻⁴ For example, specific receptors for 1,25(OH)₂D₃ are present in normal human monocytes and in activated lymphocytes.²⁰⁻²¹ The observations that synovial fluid macrophages synthesise 1,25(OH)₂D₃ and that both peripheral blood lymphocytes²² and synovial tissue derived fibroblasts²³ from patients with rheumatoid arthritis express its receptors is consistent with 1,25(OH)₂D₃ also having an immunological part to play in joint disease. Furthermore, many of the reported receptor mediated effects of 1,25(OH)₂D₃ may be relevant to arthritis; these include inhibition of both B and T lymphocyte proliferation and interleukin 2 production by T lymphocytes.^{3-4, 24} 1,25(OH)₂D₃ also promotes differentiation of monocytes into macrophages and the fusion of macrophages to multinucleated giant cells with bone resorbing activity.²⁵⁻²⁶ This is in addition to the known ability of 1,25(OH)₂D₃ to promote osteoclastic bone resorption and to inhibit osteoblast collagen synthesis.²⁷

Prostaglandins, particularly prostaglandin E₁ and prostaglandin E₂ secreted by polymorphs, monocyte macrophages and fibroblasts,^{18-19, 28-29} are believed to play a primary part in acute arthritic inflammatory episodes in two ways. Firstly, prostaglandin E₂ affects the increased vascular permeability which allows the entry of proteins, albumin, and fibrinogen into the joint and synovial fluid. Secondly, prostaglandin E₂ has been shown to inhibit various aspects of the immune response, including lymphocyte proliferation, lymphokine secretion, macrophage collagenase synthesis, and the activity of natural killer cells and macrophages.³⁰ Use of prostaglandin synthesis inhibitors, however, has indicated that prostaglandins may not play a significant part in the long term progression of arthritis.¹⁸ If this is so then prostaglandin E₂ may inhibit 1,25(OH)₂D₃ synthesis during acute inflammatory episodes when concentrations are increased. During the quiescent phase of the disease, however, there may still be enough activated macrophages present in the synovium that can synthesise 1,25(OH)₂D₃.¹⁶ This may be important for immune function and it may also promote bone resorption and contribute to the development of the localised periarticular osteoporosis that is associated with chronic arthritis.³¹⁻³² Control of 1,25(OH)₂D₃ synthesis by prostaglandins, cytokines, drugs, and free radicals known to be present within the local microenvironment of the diseased joint, and elucidation of the mode of action of 1,25(OH)₂D₃ in the arthritic joint clearly warrant further study.

NJ, USA) kindly provided the standards for 25(OH)D₃, 24,25(OH)₂D₃, and 1,25(OH)₂D₃.

- Haussler M R, McCain T A. Basic and clinical concepts related to vitamin D metabolism and action. *N Engl J Med* 1977; **297**: 974-83.
- Reichel H, Koeffler H P, Norman A W. The role of the vitamin D endocrine system in health and disease. *N Engl J Med* 1989; **320**: 980-91.
- Tsoukas C D, Provvedini D M, Manolagas S C. 1,25-Dihydroxyvitamin D₃: a novel immunoregulatory hormone. *Science* 1984; **224**: 1438-40.
- Rigby W F C. The immunobiology of vitamin D. *Immunobiology Today* 1988; **9**: 54-8.
- Adams J S, Sharma O P, Gacad M A, Singer F R. Metabolism of 25-hydroxyvitamin D₃ by cultured pulmonary alveolar macrophages in sarcoidosis. *J Clin Invest* 1983; **72**: 1856-60.
- Cadranel J, Hance A J, Milleron B, Paillard F, Akoun G M, Garabedian M. Vitamin D metabolism in tuberculosis: production of 1,25(OH)₂D₃ by cells recovered by bronchoalveolar lavage and the role of this metabolite in calcium homeostasis. *Am Rev Respir Dis* 1988; **138**: 984-9.
- Hayes M E, Denton J, Freemont A J, Mawer E B. Synthesis of the active metabolite of vitamin D, 1,25(OH)₂D₃, by synovial fluid macrophages in arthritic disease. *Ann Rheum Dis* 1989; **48**: 723-9.
- Hayes M E, Bayley D, Still P, *et al*. Differential metabolism of 25-hydroxyvitamin D₃ by cultured synovial fluid macrophages and fibroblast-like cells from patients with arthritis. *Ann Rheum Dis* 1992; **51**: 220-6.
- Hayes M E, O'Donoghue D J O, Ballardie F W, Mawer E B. Peritonitis induces the synthesis of 1 α ,25-dihydroxyvitamin D₃ in macrophages from CAPD patients. *FEBS Lett* 1987; **220**: 307-10.
- Hayes M E, Bayley D, Drayson M, Freemont A J, Denton J, Davies M, Mawer E B. Metabolism of 25-hydroxyvitamin D₃ by blood derived macrophages from a patient with alveolar rhabdomyosarcoma during short-term culture and 1 α ,25-dihydroxyvitamin D₃ after long-term culture. *J Steroid Biochem Mol Biol* 1991; **38**: 301-5.
- Koeffler H P, Reichel H, Bishop J E, Norman A W. Gamma-interferon stimulates production of 1,25-dihydroxyvitamin D₃ by normal human macrophages. *Biochem Biophys Res Commun* 1985; **127**: 596-603.
- Reichel H, Koeffler P, Bishop J E, Norman A W. 25-hydroxyvitamin D₃ metabolism by lipopolysaccharide-stimulated normal human macrophages. *J Clin Endocrinol Metab* 1987; **64**: 1-9.
- Pryke A M, Duggan C, White C P, Posen S, Mason R S. Tumour necrosis factor alpha induces vitamin D-1-hydroxylase activity in normal human alveolar macrophages. *J Cell Physiol* 1990; **142**: 652-6.
- Sandler L M, Winearls C G, Fraher L J, Clemens T L, Smith R, O'Riordan J L H. Studies of the hypercalcaemia of sarcoidosis: effect of steroids and exogenous vitamin D₃ on circulating concentrations of 1,25-dihydroxy vitamin D₃. *Q J Med* 1984; **210**: 165-80.
- Davies M, Mawer E B, Hayes M E, Lumb G A. Abnormal vitamin D metabolism in Hodgkin's lymphoma. *Lancet* 1985; **i**: 1186-8.
- Mawer E B, Hayes M E, Still P E, *et al*. Evidence for non-renal synthesis of 1,25-dihydroxyvitamin D in patients with inflammatory arthritis. *J Bone Miner Res* 1991; **6**: 733-9.
- Wark J D, Larkins R G, Eisman J A, Wilson K R. Regulation of 25-hydroxyvitamin-D-1 α -hydroxylase in chick isolated renal tubules: effects of prostaglandin E₂, frusemide and acetylsalicylate. *Clin Sci* 1981; **62**: 53-9.
- Hopkins S J. Cytokines and eicosinoids in rheumatic diseases. *Ann Rheum Dis* 1990; **49**: 207-11.
- Fidler I J, Nii A, Utsugi T, Brown D, Bakouche O, Kleinerman E S. Differential release of TNF- α , IL1 and PGE₂ by human blood monocytes subsequent to interaction with different bacterial derived agents. *Lymphokine Res* 1990; **9**: 449-63.
- Provvedini D M, Tsoukas C D, Deftos L J, Manolagas S C. 1,25-dihydroxyvitamin D₃ receptors in human leukocytes. *Science* 1983; **221**: 1181-3.
- Bhalla A K, Amento E P, Clemens T L, Holick M F, Krane S M. Specific high-affinity receptors for 1,25-dihydroxyvitamin D₃ in human peripheral blood mononuclear cells: presence in monocytes and induction on T lymphocytes following activation. *J Clin Endocrinol Metab* 1983; **57**: 1308-10.
- Manolagas S C, Wernitz D A, Tsoukas C D, Provvedini D M, Vaughan J H. 1,25-dihydroxyvitamin D₃ receptors in lymphocytes from patients with rheumatoid arthritis. *J Lab Clin Med* 1986; **108**: 596-600.
- Sambrook P N, Eisman J A, Brett S. 1,25-Dihydroxyvitamin D₃ receptors in synovial cells in rheumatoid arthritis [abstract]. *Aust NZ J Med* 1987; **17**: 120.
- Shiozawa S, Shiozawa K, Tanaka Y, Fujita T. 1 α ,25-Dihydroxyvitamin D₃ inhibits proliferative response of T- and B-lymphocytes in serum free culture. *Int J Immunopharmacol* 1987; **9**: 719-23.
- Abe E, Miyaura C, Tanaka H, *et al*. 1 α ,25-Dihydroxyvitamin D₃ promotes fusion of mouse alveolar macrophages both by direct mechanisms and by spleen cell-mediated indirect mechanisms. *Proc Natl Acad Sci USA* 1983; **80**: 5583-7.
- Bar-Shavit Z, Teitelbaum S L, Reitsma P, *et al*. Induction of monocytic differentiation and bone resorption by 1,25-

- dihydroxyvitamin D₃. *Proc Natl Acad Sci USA* 1983; **80**: 5907–11.
- 27 Raisz L G, Kream B E, Smith M A, Simmons H A. Comparison of the effects of vitamin D metabolites on collagen synthesis and resorption of fetal rat bone in organ culture. *Calcif Tissue Int* 1980; **32**: 135–8.
- 28 Goldyne M C. Eicosanoid metabolism by lymphocytes: do all human nucleated cells generate eicosanoids. *Pharmacol Res* 1989; **21**: 241–5.
- 29 Akama H, Ichikawa Y, Matsushita Y, Shinozawa T, Homma M. Mononuclear cells enhance prostaglandin E₂ production of polymorphonuclear leukocytes via tumor necrosis factor. *Biochem Biophys Res Commun* 1990; **168**: 857–62.
- 30 Wan J M F, Haw M P, Blackburn G L. Nutrition, immune function, and inflammation: an overview. *Proc Nutr Soc* 1989; **48**: 315–35.
- 31 Sambrook P N, Reeve J. Bone disease in rheumatoid arthritis. *Clin Sci* 1988; **74**: 225–30.
- 32 Sambrook P N, Shawe D, Hesp R, *et al*. Rapid periarticular bone loss in rheumatoid arthritis: possible promotion by normal circulating concentrations of parathyroid hormone or calcitriol (1,25-dihydroxyvitamin D₃). *Arthritis Rheum* 1990; **33**: 615–22.