Available online at www.sciencedirect.com
ScienceDirect
Journal of Steroid Biochemistry & Molecular Biology 103 (2007) 316–321
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Extra-renal 25-hydroxyvitamin D_3-1α-hydroxylase in human health and disease

Martin Hewison [a,*], Fiona Burke [b], Katie N. Evans [b], David A. Lammas [b], David M. Sansom [b], Philip Liu [c], Robert L. Modlin [c], John S. Adams [a]

[a] *Burns and Allen Research Institute, Division of Endocrinology, Cedars-Sinai Medical Center and David Geffen School of Medicine at UCLA, Los Angeles, CA 90048, USA*
[b] *Division of Immunity and Infection, Institute of Biomedical Research, The University of Birmingham Medical School, Birmingham B15 2TT, UK*
[c] *Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA*

Abstract

Although ectopic expression of 25-hydroxyvitamin D_3-1α-hydroxylase (1α-OHase) has been recognized for many years, the precise function of this enzyme outside the kidney remains open to debate. Three specific aspects of extra-renal 1α-OHase have attracted most attention: (i) expression and regulation in non-classical tissues during normal physiology; (ii) effects on the immune system and inflammatory disease; (iii) expression and function in tumors. The most well-recognized manifestation of extra-renal 1α-OHase activity remains that found in some patients with granulomatous diseases where locally synthesized $1\alpha,25(OH)_2D_3$ has the potential to spill-over into the general circulation. However, immunohistochemistry and mRNA analyses suggest that 1α-OHase is also expressed by a variety of normal human tissues including the gastrointestinal tract, skin, vasculature and placenta. This has promoted the idea that autocrine/paracrine synthesis of $1,25(OH)_2D_3$ contributes to normal physiology, particularly in mediating the potent effects of vitamin D on innate (macrophage) and acquired (dendritic cell) immunity. We have assessed the capacity for synthesis of $1,25(OH)_2D_3$ in these cells and the functional significance of autocrine responses to 1α-hydroxylase. Data suggest that local synthesis of $1,25(OH)_2D_3$ may be a preferred mode of response to antigenic challenge in many tissues.

Keywords: Vitamin D; Extra-renal; 1α-Hydroxylase; 24-Hydroxylase; Granulomatous; Macrophage; Dendritic cell; Crohn's disease; Tuberculosis; Cancer

1. Introduction

Synthesis of the active form of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), from its precursor form, 25-hydroxyvitamin D_3 ($25OHD_3$) is a pivotal step in calcium homeostasis and bone metabolism. Not only does this represent the point at which the major circulating form of vitamin D ($25OHD_3$) becomes a hormone, but it is also the point at which peptide caiciotropic factors such as parathyroid hormone interact with vitamin D physiology [1]. As a consequence, caiciotropic synthesis of $1,25(OH)_2D_3$ by the kidney, catalyzed by the enzyme 25-hydroxyvitamin D_3-1α-hydroxylase (1α-OHase) is tightly regulated with feedback control mechanisms that include a dedicated catabolic enzyme, 24-hydroxylase (24-OHase) [2,3]. As our awareness of the pluripotency of $1,25(OH)_2D_3$ has increased, a key question that has arisen concerns the role of 1α-OHase outside the kidney and the extent to which this represents part of normal physiology. Here we have used *in vitro* models to assess the relative functional efficacy of exogenous versus endogenous $1,25(OH)_2D_3$ in modulating immune responses. We also discuss the significance of this with respect to other models of extra-renal 1α-hydroxylase

* Corresponding author at: Room D-3088, Burns and Allen Research Institute, Division of Endocrinology, Cedars-Sinai Medical Center and David Geffen School of Medicine at UCLA, Los Angeles, CA 90048, USA.
Tel.: +44 121 414 3776; fax: +44 121 414 7610.
E-mail address: M.Hewison@bham.ac.uk (M. Hewison).

0960-0760/$ – see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.jsbmb.2006.12.078

activity and diseases in which there is dysregulated extra-renal synthesis of $1,25(OH)_2D_3$.

2. Materials and methods

2.1. Generation of monocyte-derived dendritic cells and maxcrophages

Monocyte-derived dendritic cells (DCs) and macrophages were derived from buffy coats of healthy donors by density centrifugation over Lymphoprep (Robbins Scientific, Sunnyvale, CA, USA), resuspended in RPMI supplemented with 5% human AB serum (HD supplies, Buckinghamshire, UK) and allowed to adhere to plastic at 37 °C for 2 h. Non-adherent cells were then removed and monocytes cultured in RPMI supplemented with the following: 5% AB serum; 2 mM L-glutamine; 100 μg/ml streptomycin; 100 μg/ml penicillin; 800 μg/ml GM-CSF (Sandoz Pharmaceuticals). Dendritic cells cultures also received 1000 μg/ml IL-4 (R&D systems, Abingdon UK) in 75 cm^2 flasks. 1, $25(OH)_2D_3$ (100 nM) or $25(OH)D_3$ (5–150 nM) (a kind gift from Dr. Lise Binderup, Leo Pharmaceuticals, Ballerup, Denmark), as well as ketoconazole (5 μM) (Sigma, Poole, UK) were added on day 5 of DC/macrophage culture and analysis of vitamin D metabolites and flow cytometry carried out two days later.

2.2. Analysis of $1,25(OH)_2D_3$ in DC and macrophage cell cultures

The accumulation of $1,25(OH)_2D_3$ in cultures of DCs or macrophages incubated with precursor $25OHD_3$ was carried out using a specific radioimmunoassay kit as described by the manufacturers (IDS, Boldon, UK). Data were reported as concentration of $1,25(OH)_2D_3$ (pM) ± standard deviation ($n = 3$).

2.3. Analysis of DC and macrophage marker expression by fluorescence-activated cell sorting (FACS)

Expression of CD83, CD86, HLA-DR (DCs) and CD14 (macrophages) was carried out as described previously [4]. Data were reported as the percentage change in marker expression relative to vehicle-treated control cells ± standard deviation ($n = 3$).

3. Results

Human monocyte-derived DCs and macrophages actively synthesize $1,25(OH)_2D_3$ *in vitro*. To assess the magnitude of this with respect to autocrine responses to $25OHD_3$ in these cells we measured the accumulated levels of $1,25(OH)_2D_3$ in day 5 cultures of DC or macrophages incubated for 48 h with varying levels of $25OHD_3$. Preliminary studies indicated that levels of $25OHD_3$ in cultures containing 5% AB serum were approximately 5 nM (data not shown). We therefore supplemented DC and macrophage cultures with vehicle, or 45–145 nM $25OHD_3$ to adjust final culture concentrations to 5, 50 and 150 nM.

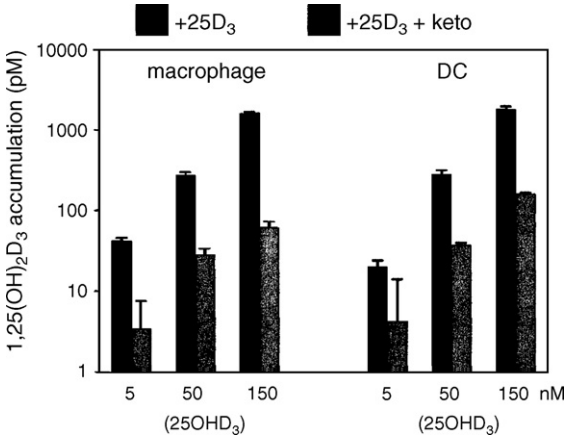

Fig. 1. Accumulation of $1,25(OH)_2D_3$ in cultures of dendritic cells (DCs) and macrophages incubated with $25OHD_3$. Monocyte-derived DCs and macrophages were cultured for 5 days and then incubated with various levels of exogenous unlabelled $25OHD_3$ to give final concentrations of 5, 50 or 150 nM. Supernatants from the cells were then assayed for levels of $1,25(OH)_2D_3$ by radioimmunoassay. Data are the mean ± S.D. of $n = 3$ observations.

These values were chosen to represent conditions of vitamin D deficiency, vitamin D insufficiency and vitamin D sufficiency, respectively. Results shown in Fig. 1 indicated that unsupplemented DCs and macrophages were exposed to between 20 and 40 nM $1,25(OH)_2D_3$. At least some of this was due to cellular metabolism of $25OHD_3$ rather than endogenous hormone as co-culture with the 1α-OHase inhibitor ketoconazole suppressed $1,25(OH)_2D_3$ levels. Supplementary $25OHD_3$ increased levels of $1,25(OH)_2D_3$ in both DC and macrophage cultures in a dose-dependent fashion with the 150 nM $25OHD_3$ generating levels of $1,25(OH)_2D_3$ between 2 and 3 nM. Once again this was suppressed by co-culture with ketoconazole implicating DCs or macrophages as the primary source of $1,25(OH)_2D_3$.

DCs and macrophages cultured in the presence of added $25OHD_3$ were then assayed to determine whether or not the $1,25(OH)_2D_3$ generated in Fig. 1 was sufficient to elicit autocrine responses. Data in Fig. 2A showed that even at 50 nM the added $25OHD_3$ was able to significantly increase CD14 expression in macrophages and this effect was diminished in the presence of ketoconazole endorsing an autocrine mode of action. Cells were also treated with $1,25(OH)_2D_3$ to compare responses between endogenously synthesized and exogenously added hormone. Data indicated that 150 nM $25OHD_3$ was as effective as 100 nM $1,25(OH)_2D_3$ in stimulating CD14 expression despite the fact that this mode of supplementation generated less than 5 nM $1,25(OH)_2D_3$ in either DC or macrophage cultures (Fig. 1). A similar pattern was also observed for the DC markers CD83, CD86 and

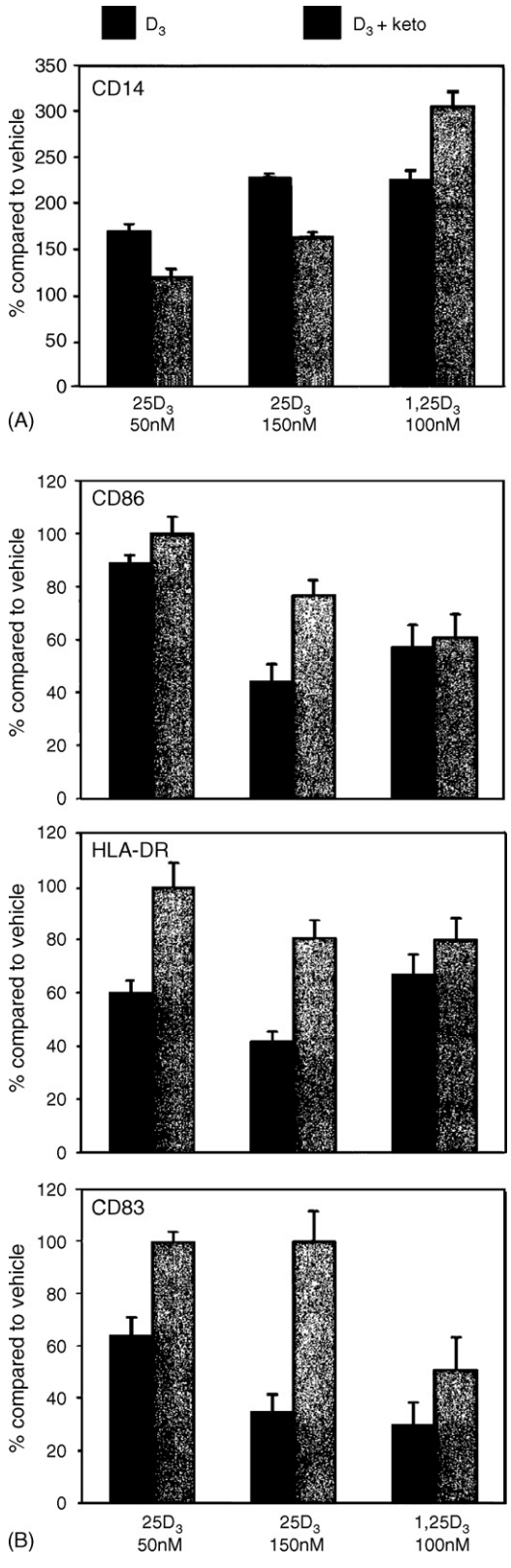

Fig. 2. Functional responses to endogenous $1,25(OH)_2D_3$ production in DCs and macrophages. Monocyte-derived DCs and macrophages were cultured for 5 days and then incubated with various levels of exogenous unlabelled $25OHD_3$ to give final concentrations of 5, 50 or 150 nM. Cells were then subjected to FACS analysis for the following cell surface antigens: (A) CD14 (macrophages); (B) CD83, CD86, HLA-DR (DCs). Data are presented as % change in expression relative to 5 nM $25OHD_3$ cultures. Values are mean ± S.D. of $n = 3$ observations.

HLA-DR which were all suppressed by treatment with either $25OHD_3$ or $1,25(OH)_2D_3$ (Fig. 2B).

4. Discussion

Extra-renal synthesis of $1,25(OH)_2D_3$ was first documented more than quarter of a century ago following studies of vitamin D metabolism in human pregnancy [5] and the granulomatous disease sarcoidosis [6]. This raised three important questions: (i) Is extra-renal 1α-OHase a different enzyme to that found in the kidney? (ii) Is extra-renal 1α-OHase primarily a feature of human disease rather than normal physiology? (iii) Does extra-renal 1α-OHase manifest itself by changes in systemic $1,25(OH)_2D_3$ as occurs in the kidney, or does this instead reflect the autocrine/paracrine actions of vitamin D? The first of these questions was resolved following the cloning of the human gene for 1α-OHase (CYP27b1) which revealed indentical cDNA sequences in renal [7] and extra-renal tissues [8]. The key issue concerning the extent to which extra-renal expression of 1α-OHase is a component of normal vitamin D physiology has been harder to resolve but has recently attracted much attention with the recognition that vitamin D status may influence susceptibility to many common human diseases [9]. Specifically, it has been proposed that individual vitamin D status is most readily reflected by circulating levels of $25OHD_3$ rather than $1,25(OH)_2D_3$, and this in turn will have a greater impact on extra-renal vitamin D metabolism. Three other lines of evidence support the proposed role for extra-renal 1α-OHase in mediating the protective effects of $1,25(OH)_2D_3$ on human disease: (i) the dysregulation of $1,25(OH)_2D_3$ production with human disease *in vivo*; (ii) the tissue distribution of 1α-OHase; (iii) the autocrine actions of 1α-OHase *in vitro*. These issues will be highlighted in the remainder of the discussion.

4.1. Extra-renal 1α-hydroxylase and granulomatous disease

Following the initial reports of increased $1,25(OH)_2D_3$ production in patients with sarcoidosis, similar evidence for dysregulated extra-renal 1α-OHase activity has been presented for more than twenty other granulomatous diseases [10]. In most cases the enhanced synthesis of $1,25(OH)_2D_3$ is associated with varying degrees of hypercalcemia or hypercalciuria but may also have additional complications. For example, we recently described elevated serum $1,25(OH)_2D_3$ levels in patients with Crohn's disease and enhanced expression of 1α-OHase in affected tissue [11]. Here circulating levels of the hormone were not as great as observed in many sarcoidosis patients but were still significantly higher than

control populations, and were associated with hypercalciuria rather than hypercalcemia. Importantly, analysis of bone mineral density showed that the bone loss which is a prevalent complication of Crohn's disease was independently associated with serum $1,25(OH)_2D_3$ levels [11]. Further analysis of vitamin D metabolism in inflammatory bowel disease may play a key role in clarifying the physiological relevance of extra-renal 1α-OHase. This is because although patients with Crohn's disease synthesize more extra-renal $1,25(OH)_2D_3$, it is clear that many are also vitamin D deficient [12]. Further studies are required to determine whether or not this paradox is a consequence of gastrointestinal abnormality or whether vitamin D deficiency contributes to the onset of disease. However, as extra-renal 1α-OHase contributes to some of the complications of Crohn's disease, it is not unreasonable to hypothesize that it may also contribute to disease susceptibility via a similar mechanism. It also seems likely that this may also occur with related diseases such as tuberculosis, where vitamin D deficiency is associated with the onset of disease [13], but where hypercalcemia and elevated $1,25(OH)_2D_3$ may be a feature of advanced disease [14].

4.2. Extra-renal 1α-hydroxylase and cancer

In addition to granulomatous diseases, we have also reported dysregulated extra-renal 1α-OHase expression and activity associated with B-cell lymphoma, dysgerminomas and breast cancer [15–17]. In lymphomas and dysgerminomas, the increased $1,25(OH)_2D_3$ production was associated with hypercalcemia in a similar fashion to that seen in sarcoidosis patients [16,17]. Increased capacity for synthesis of $1,25(OH)_2D_3$ was also observed in breast tumors, but was accompanied in this case by enhanced 24-OHase activity suggesting that in this type of neoplasm there is attenuation of $1,25(OH)_2D_3$ accumulation via an autocrine catabolic pathway. Furthermore, localization of 1α-OHase revealed that the enzyme was strongly expressed by immune infiltrates in all three types of tumor, although this was more evident in the lymphoma and dysgerminomas which characteristically have significant immune involvement. Inflammatory signaling may also influence vitamin D metabolism and function in the absence of immune cell infiltration. Recent studies have shown that epithelial cell toll-like receptors (TLRs) play an important role in maintaining normal tissue homeostasis [18], and dysregulation of this mechanism may contribute to tumor development. The presence of 1α-OHase in tumor-adjacent macrophages may also help to explain the paradoxical loss of 1α-OHase activity seen with epithelial cancer cells *in vitro* [19]. Specifically, it is unclear whether the reported loss of 1α-OHase activity in these cells is a factor contributing to the development of the tumor, or is instead a consequence of culture selection. It also seems likely that different tumors will express and utilize extra-renal 1α-OHase in different ways. This is perhaps best illustrated by the varying levels of expression for 1α-hydroxylase in different stages of colon [20] and prostate cancer [21].

4.3. Distribution of extra-renal 1α-hydroxylase in normal human tissue

Although the expression, activity and pathophysiological effects of extra-renal 1α-OHase have been well characterized for human diseases, the function of the enzyme in extra-renal tissues during normal physiology remains less clear. Previously reported *in situ* hybridization and immunohistochemistical analyses by our group have suggest that 1α-OHase is more widely distributed than previously thought, with the enzyme detectable in skin, placenta, colon, pancreas, vasculature and parts of the brain [22–24]. Although many of these observations have been endorsed by other laboratories, recent studies using a CYP27b1 promoter-reporter mouse have sounded a note of caution [25]. In this model CYP27b1 promoter activity was only detectable in the renal cortex and placenta and was notably absent from keratinocytes, the source of mRNA for the original human 1α-OHase cDNA clone. Further studies are required to determine whether or not this is a true reflection of 1α-OHase expression, particularly as the reporter LacZ gene used in the model is incorporated into the genome at the expense of the mouse CYP27b1 gene itself, and can only be detected in animals on vitamin D and calcium-depleted diets. The latter may be particularly important as in some tissues 1α-OHase expression appears to be more closely linked to expression of immune activators such as TLRs rather than calciotropic factors [26]. Consequently, in the absence of an appropriate TLR signal, expression of 1α-OHase may be limited.

4.4. Functional impact of extra-renal $1,25(OH)_2D_3$ production

The presence of 1α-OHase in extra-renal tissues has been proposed as a mechanism by which vitamin D may exert a raft of protective actions within these tissues. We have previously referred to this as 'barrier function' to include possible antiproliferative, barrier integrity and immunomodulatory effects by both epithelial, endothelial and immune cells [27]. Within this broad grouping of cells and effects, the most robust functional response to extra-renal 1α-OHase activity is seen with macrophages and DCs which both show potent upregulation of 1α-OHase expression and activity as they differentiate towards a mature phenotype [4,28]. Thus, both DCs and macrophages are readily able to synthesize $1,25(OH)_2D_3$, with potential autocrine and paracrine consequences. In DCs localized production of $1,25(OH)_2D_3$ may affect proliferation and phenotype of adjacent T-cells but it is also able to exert autocrine effects by suppressing DC maturation and maintaining these cells in a more tolerogenic state [27]. Like DCs, macrophages are also able to present antigen and influence acquired immune responses but we have recently shown that the most likely function of 1α-hydroxylase in these cells is to stimulate innate immunity [29]. In this study expression of 1α-OHase by

monocytes was induced as a result of ligand binding to TLR2 but the enzyme was only functional if sufficient substrate ($25OHD_3$) was available for conversion to $1,25(OH)_2D_3$. This was further emphasized by experiments which showed that macrophages cultured in serum from vitamin D-deficient African-Americans were less able to induce the antimicrobial peptide cathelicidin than cells grown with Caucasian serum, and that this could be corrected by supplementation of the African-American serum with $25OHD_3$.

There are many questions concerning extra-renal 1α-OHase that remain unanswered. These include the key issue of why extra-renal 1α-OHase becomes dysregulated in granulomatous diseases and some cancers. Is this due to initial vitamin D deficiency leading to abnormal TLR signaling and persistence of inflammation or tumor development? One recent study has shown that $1,25(OH)_2D_3$ suppresses expression of TLR2 and TLR4, raising the exciting possibility that in addition to its antibacterial effects, $1,25(OH)_2D_3$ plays a role in feedback regulation of TLR signaling [30]. Another important question concerns the apparent resistance to $1,25(OH)_2D_3$ in tumors and affected inflammatory tissue. In the case of the latter this may be due to dysregulation of vitamin D signaling in the presence of specific inflammatory cytokines [31]. However, we have also recently shown that regulation of vitamin D responses via expression of the catabolic enzyme 24-OHase is different in macrophages, being associated primarily with a splice-variant form of the enzyme that appears to act as a non-functional decoy with the capacity to bind both $25OHD_3$ and $1,25(OH)_2D_3$ [32]. Finally, a question that is fundamental to all tissues that express 1α-OHase is the extent to which this is reflected by actual synthesis of $1,25(OH)_2D_3$ *in vivo*. In the kidney the acquisition of substrate $25OHD_3$ for conversion to $1,25(OH)_2D_3$ is facilitated by a specific interaction between the vitamin D binding protein and the LDL-like receptor megalin [33]. In future studies it will be interesting to determine whether similar mechanisms are required at an *in vivo* level to achieve efficient extra-renal synthesis of $1,25(OH)_2D_3$.

References

[1] J.L. Omdahl, E.A. Bobrovnikova, S. Choe, P.P. Dwivedi, B.K. May, Overview of regulatory cytochrome P450 enzymes of the vitamin D pathway, Steroids 66 (3–5) (2001) 381–389.

[2] R. Bland, D. Zehnder, M. Hewison, Expression of 25-hydroxyvitamin D_3-1α-hydroxylase along the nephron: new insights into renal vitamin D metabolism, Curr. Opin. Nephrol. Hypertens. 9 (1) (2000) 17–22.

[3] D. Zehnder, M. Hewison, The renal function of 25-hydroxyvitamin D_3-1α-hydroxylase, Mol. Cell. Endocrinol. 151 (1–2) (1999) 213–220.

[4] M. Hewison, L. Freeman, S.V. Hughes, K.N. Evans, R. Bland, A.G. Eliopoulos, M.D. Kilby, P.A. Moss, R. Chakraverty, Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells, J. Immunol. 170 (11) (2003) 5382–5390.

[5] T.K. Gray, G.E. Lester, R.S. Lorenc, Evidence for extra-renal 1α-hydroxylation of 25-hydroxyvitamin D_3 in pregnancy, Science 204 (4399) (1979) 1311–1313.

[6] G.L. Barbour, J.W. Coburn, E. Slatopolsky, A.W. Norman, R.L. Horst, Hypercalcemia in an anephric patient with sarcoidosis: evidence for extrarenal generation of 1,25-dihydroxyvitamin D, N. Engl. J. Med. 305 (8) (1981) 440–443.

[7] T. Monkawa, T. Yoshida, S. Wakino, T. Shinki, H. Anazawa, H.F. Deluca, T. Suda, M. Hayashi, T. Saruta, Molecular cloning of cDNA and genomic DNA for human 25-hydroxyvitamin D_3 1α-hydroxylase, Biochem. Biophys. Res. Commun. 239 (2) (1997) 527–533.

[8] G.K. Fu, D. Lin, M.Y. Zhang, D.D. Bikle, C.H. Shackleton, W.L. Miller, A.A. Portale, Cloning of human 25-hydroxyvitamin D-1α-hydroxylase and mutations causing vitamin D-dependent rickets type 1, Mol. Endocrinol. 11 (13) (1997) 1961–1970.

[9] W.B. Grant, Epidemiology of disease risks in relation to vitamin D insufficiency, Prog. Biophys. Mol. Biol. (2006).

[10] M. Hewison, J.S. Adams, Hypercalcemia Due to Granuloma-Forming Disorders Primer on Metabolic Bone Diseases, 5th ed., Lippincott, Williams and Wilkins, New York, 2003, pp. 257–259.

[11] M.T. Abreu, V. Kantorovich, E.A. Vasiliauskas, U. Gruntmanis, R. Matuk, K. Daigle, S. Chen, D. Zehnder, Y.C. Lin, H. Yang, M. Hewison, J.S. Adams, Measurement of vitamin D levels in inflammatory bowel disease patients reveals a subset of Crohn's disease patients with elevated 1,25-dihydroxyvitamin D and low bone mineral density, Gut 53 (8) (2004) 1129–1136.

[12] K.V. Haderslev, P.B. Jeppesen, H.A. Sorensen, P.B. Mortensen, M. Staun, Vitamin D status and measurements of markers of bone metabolism in patients with small intestinal resection, Gut 52 (5) (2003) 653–658.

[13] A. Ustianowski, R. Shaffer, S. Collin, R.J. Wilkinson, R.N. Davidson, Prevalence and associations of vitamin D deficiency in foreign-born persons with tuberculosis in London, J. Infect. 50 (5) (2005) 432–437.

[14] P.J. Gkonos, R. London, E.D. Hendler, Hypercalcemia and elevated 1,25-dihydroxyvitamin D levels in a patient with end-stage renal disease and active tuberculosis, N. Engl. J. Med. 311 (26) (1984) 1683–1685.

[15] K. Townsend, C.M. Banwell, M. Guy, K.W. Colston, J.L. Mansi, P.M. Stewart, M.J. Campbell, M. Hewison, Autocrine metabolism of vitamin D in normal and malignant breast tissue, Clin. Cancer Res. 11 (9) (2005) 3579–3586.

[16] K.N. Evans, H. Taylor, D. Zehnder, M.D. Kilby, J.N. Bulmer, F. Shah, J.S. Adams, M. Hewison, Increased expression of 25-hydroxyvitamin D-1α-hydroxylase in dysgerminomas: a novel form of humoral hypercalcemia of malignancy, Am. J. Pathol. 165 (3) (2004) 807–813.

[17] M. Hewison, V. Kantorovich, H.R. Liker, A.J. Van Herle, P. Cohan, D. Zehnder, J.S. Adams, Vitamin D-mediated hypercalcemia in lymphoma: evidence for hormone production by tumor-adjacent macrophages, J. Bone Miner. Res. 18 (3) (2003) 579–582.

[18] S. Rakoff-Nahoum, J. Paglino, F. Eslami-Varzaneh, S. Edberg, R. Medzhitov, Recognition of commensal micro flora by toll-like receptors is required for intestinal homeostasis, Cell 118 (2) (2004) 229–241.

[19] J.Y. Hsu, D. Feldman, J.E. McNeal, D.M. Peehl, Reduced lalpha-hydroxylase activity in human prostate cancer cells correlates with decreased susceptibility to 25-hydroxyvitamin D_3-induced growth inhibition, Cancer Res. 61 (7) (2001) 2852–2856.

[20] H.S. Cross, G. Bises, D. Lechner, T. Manhardt, E. Kallay, The vitamin D endocrine system of the gut—its possible role in colorectal cancer prevention, J. Steroid Biochem. Mol. Biol. 97 (1–2) (2005) 121–128.

[21] T.C. Chen, L. Wang, L.W. Whitlatch, J.N. Flanagan, M.F. Holick, Prostatic 25-hydroxyvitamin D-1α-hydroxylase and its implication in prostate cancer, J. Cell. Biochem. 88 (2) (2003) 315–322.

[22] D. Zehnder, R. Bland, M.C. Williams, R.W. McNinch, A.J. Howie, P.M. Stewart, M. Hewison, Extrarenal expression of 25-hydroxyvitamin D_3-1α-hydroxylase, J. Clin. Endocrinol. Metab. 86 (2) (2001) 888–894.

[23] D. Zehnder, R. Bland, R.S. Chana, D.C. Wheeler, A.J. Howie, M.C. Williams, P.M. Stewart, M. Hewison, Synthesis of 1,25-dihydroxyvitamin D_3 by human endothelial cells is regulated by inflammatory cytokines: a novel autocrine determinant of vascular cell adhesion, J. Am. Soc. Nephrol. 13 (3) (2002) 621–629.

[24] D. Zehnder, K.N. Evans, M.D. Kilby, J.N. Bulmer, B.A. Innes, P.M. Stewart, M. Hewison, The ontogeny of 25-hydroxyvitamin D_3 1α-hydroxylase expression in human placenta and decidua, Am. J. Pathol. 161 (1) (2002) 105–114.

[25] J.L. Vanhooke, J.M. Prahl, C. Kimmel-Jehan, M. Mendelsohn, E.W. Danielson, K.D. Healy, H.F. Deluca, CYP27B1 null mice with LacZreporter gene display no 25-hydroxyvitamin D_3-1α-hydroxylase promoter activity in the skin, Proc. Natl. Acad. Sci. USA 103 (1) (2006) 75–80.

[26] K. Townsend, K.N. Evans, M.J. Campbell, K.W. Colston, J.S. Adams, M. Hewison, Biological actions of extra-renal 25-hydroxyvitamin D-1α-hydroxylase and implications for chemoprevention and treatment, J. Steroid Biochem. Mol. Biol. 97 (1–2) (2005) 103–109.

[27] M. Hewison, D. Zehnder, R. Chakraverty, J.S. Adams, Vitamin D and barrier function: a novel role for extra-renal 1α-hydroxylase, Mol. Cell Endocrinol. 215 (1–2) (2004) 31–38.

[28] M. Kreutz, R. Andreesen, S.W. Krause, A. Szabo, E. Ritz, H. Reichel, 1,25-Dihydroxyvitamin D_3 production and vitamin D3 receptor expression are developmentally regulated during differentiation of human monocytes into macrophages, Blood 82 (4) (1993) 1300–1307.

[29] P.T. Liu, S. Stenger, H. Li, L. Wenzel, B.H. Tan, S.R. Krutzik, M.T. Ochoa, J. Schauber, K. Wu, C. Meinken, D.L. Kamen, M. Wagner, R. Bals, A. Steinmeyer, U. Zugel, R.L. Gallo, D. Eisenberg, M. Hewison, B.W. Hollis, J.S. Adams, B.R. Bloom, R.L. Modlin, Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response, Science 311 (5768) (2006) 1770–1773.

[30] K. Sadeghi, B. Wessner, U. Laggner, M. Ploder, D. Tamandl, J. Friedl, U. Zugel, A. Steinmeyer, A. Pollak, E. Roth, G. Boltz-Nitulescu, A. Spittler, Vitamin D_3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns, Eur. J. Immunol. 36 (2) (2006) 361–370.

[31] M. Vidal, C.V. Ramana, A.S. Dusso, Stat1–vitamin D receptor interactions antagonize 1,25-dihydroxyvitamin D transcriptional activity and enhance stat1-mediated transcription, Mol. Cell Biol. 22 (8) (2002) 2777–2787.

[32] S. Ren, L. Nguyen, S. Wu, C. Encinas, J.S. Adams, M. Hewison, Alternative splicing of vitamin D-24-hydroxylase: a novel mechanism for the regulation of extrarenal 1,25-dihydroxyvitamin D synthesis, J. Biol. Chem. 280 (21) (2005) 20604–20611.

[33] A. Nykjaer, D. Dragun, D. Walther, H. Vorum, C. Jacobsen, J. Herz, F. Melsen, E.I. Christensen, T.E. Willnow, An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH)vitamin D_3, Cell 96 (4) (1999) 507–515.