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1α-Hydroxylase and innate immune responses to 25-hydroxyvitamin D in colonic cell lines☆,☆☆

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ABSTRACT

Vitamin D-insufficiency is a prevalent condition in populations throughout the world, with low serum levels of 25-hydroxyvitamin D (25OHD) linked to a variety of human health concerns including cancer, autoimmune disease and infection. Current data suggest that 25OHD action involves localized extra-renal conversion to 1,25-dihydroxyvitamin D (1,25(OH)2D) via tissue-specific expression of the enzyme 25-hydroxyvitamin D-1α-hydroxylase (1α-hydroxylase). In cells such as macrophages, expression of 1α-hydroxylase is intimately associated with toll-like receptor (TLR) recognition of pathogens. However, this mechanism may not be exclusive to extra-renal generation of 1,25(OH)2D. To investigate the relationship between TLR-mediated pathogen recognition and vitamin D-induced antibacterial activity, intracrine responses to 25OHD metabolism were explored in vitro using the established colonic cell lines Caco-2 and Caco-2 clone BBe. Analysis of antibacterial factors such as cathelicidin (LL37) and β-defensin-4 (DEFB4) was carried out following co-treatment with TLR ligands. Data indicate that, unlike macrophages, Caco-2 and BBe colonic cell lines are unresponsive to TLR-induced 1α-hydroxylase. Alternative activators of 1α-hydroxylase such as transforming growth factor β were also ineffective at priming intracrine responses to 25OHD. Thus, in common with other barrier sites such as the skin or placenta, colonic epithelial cells may require specific factors to initiate intracrine responses to vitamin D.

1. Introduction

Interaction between vitamin D and the immune system has been recognized for over a quarter of a century. Despite this, the potential importance of vitamin D to normal human innate immunity has only gained recognition in the last few years. Two key developments have underpinned this change in perspective. The first is that our perception of what constitutes adequate vitamin D status has changed. The observation that serum levels of the inactive form of vitamin D, 25-hydroxyvitamin D (25OHD) as high as 75 nM correlate inversely with parathyroid hormone [1] has prompted introduction of the term "vitamin D-insufficiency". This is defined by serum levels of 25OHD that are sub-optimal (<75 nM) but not necessarily rachitic (<20 nM) [2]. Unlike serum concentrations of active 1,25-dihydroxyvitamin D (1,25(OH)2D), which are primarily defined by renal regulators of the enzyme, 25-hydroxyvitamin D-1α-hydroxylase (1α-hydroxylase), circulating levels of 25OHD are a direct reflection of individual exposure to sunlight or dietary intake of vitamin D. As a consequence of these new parameters it is clear that the numbers of people throughout the world classified as vitamin D insufficient is much greater than originally thought [3].

The second important development to alter our perception of vitamin D concerns recent studies highlighting its potent effects on innate immunity [4–7]. Previous experiments by Rook and colleagues first demonstrated the ability of 1,25(OH)2D to suppress proliferation of pathogens such as Mycobacterium tuberculosis (M. tb) in macrophages [8]. However, it was not until much later that similar actions were described for 25OHD. In studies to define monocyte responses to M. tb, Modlin and colleagues demonstrated induction of the vitamin D receptor (VDR) and 1α-OHase in monocytes treated with ligand to TLR2/1, the principal pathogen-recognition receptor for M. tb [9]. They were then able to demonstrate activation of monocyte target genes as a consequence of local synthesis of 1,25(OH)2D from 25OHD. The key immune target of this intracrine metabolism was cathelicidin (LL37), an antimicrobial protein known to express a functional vitamin D response element in its gene promoter [10,11]. Induction of LL37 appears to be a crucial step in facilitating successful phagolyzosomal killing of bacteria, particularly M. tb [12]. Significantly, ex vivo induction of LL37 in monocytes following TLR activation

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has been shown to correlate closely with serum levels of 25OHD, underlining the importance of intracrine vitamin D metabolism to this facet of innate immunity [9,13].

Induction of LL37 by 1,25(OH)2D does not appear to be restricted to monocytes and macrophages. Similar activation of the antimicrobial factor has been reported in bronchial epithelial cells [14], myeloid cell lines [10], as well as decidual [15], and trophoblastic [16] cells of the placenta. However, this effect does not appear to be universal despite the ubiquitous expression of VDR [17]. The question therefore arises as to whether different parameters are required to promote vitamin D-induced innate immunity within different tissues. In the following manuscript we review reported mechanisms for regulation of 1α-OHase and LL37 in 'barrier' tissues such as the skin, placenta and gastrointestinal (GI) tract. In addition we present data from studies using colonic cell lines, which provide a further perspective on the role of localized synthesis of 1,25(OH)2D in promoting antimicrobial activity in cells not conventionally involved in immune responses.

2. Materials and methods

2.1. Reagents

Unlabeled 1,25-dihydroxyvitamin D_3 (1,25(OH)2D) and 25-hydroxyvitamin D_3 (25OHD) were purchased from Biomol (Plymouth Meeting, PA). Toll-like receptor (TLR) 2 ligand 19 kDa lipopeptide (19 kDa, Invivogen, San Diego, CA, USA). TLR4 ligand lipopolysaccharide (LPS, Invivogen). [^{3}H]25-hydroxyvitamin D_3 ([^{3}H]25OHD_3, specific activity, 187 Ci/mmol) was purchased from Amersham Biosciences, Piscataway, NJ.

2.2. Cell culture

The Caco-2 colonic cell line and the BBe clone of Caco-2 (both kind gifts of Dr. J.C. Fleet, Purdue University) were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FCS as described previously [18]. Cell treatments were for 24 h and included 1,25(OH)2D (10 nM), 25OHD (100 nM), 19 kDa (1 ng/ml) and LPS (100 ng/ml). Ficoll-isolated peripheral blood mononuclear cells (PBMCs) derived from anonymous donors were obtained from the Center for AIDS Research Virology Core/BSL3 Facility (supported by the National Institutes of Health award AI-28697 and by the UCLA AIDS Institute and the UCLA Council of Bioscience Resources). Monocytic cells were isolated from PBMCs by adherence and then maintained for 7 days in 24-well plates using RPMI 1640 medium supplemented with 10% FCS and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Immunex, Seattle, WA). The resulting cells showed increased expression of macrophage makers such as CD14 [19].

2.3. Quantification of 1α- and 24-hydroxylase activity

Synthesis of 1,25(OH)2D by Caco-2 and BBe cells was assessed by quantifying the conversion of radiolabeled 25OHD to 1,25(OH)2D in serum-free cultures of these cells. For each assay 50 nM [^{3}H]25OHD_3 (Amersham Biosciences, Piscataway, NJ) was added

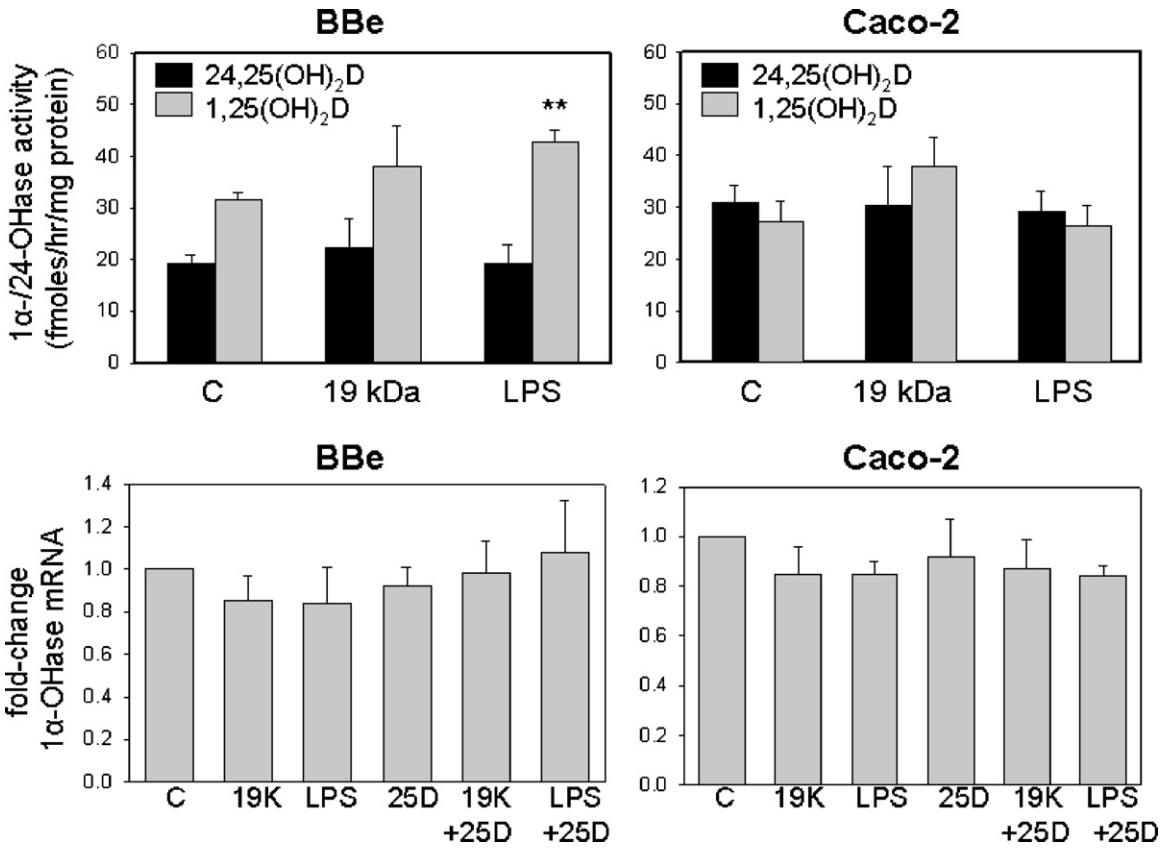

Fig. 1. Toll-like receptor regulation of vitamin D metabolism and 1α-hydroxylase expression in colonic cell lines. BBe and Caco-2 colonic cells were treated with vehicle (control, C), ligand to TLR2 (19 kDa lipoprotein, 19 kDa) or ligand to TLR4 (lipopolysaccharide, LPS), or combinations of TLR ligand with 100 nM 25-hydroxylvitamin D (25D) for 24 h and then assessed for: (A) 24-hydroxylase activity (synthesis of 24,25(OH)$_2$D) and 1α-hydroxylase activity (synthesis of 1,25(OH)2D). Data are shown as fmoles metabolite produced/h/mg protein ± SD for $n = 4$ separate assays. (B) Expression of mRNA for 1α-hydroxylase (1α-OHase). RT-PCR data are shown as fold-change in 1α-OHase mRNA relative to vehicle-treated cells. **Statistically different from vehicle-treated cells, $p < 0.01$.