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COMMENTARIES

For Whom the Bugs Toll; They Toll for D

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Commentary on: Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zugel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*. 2006 Mar 24;311(5768):1770-3.

The importance of adequate vitamin D nutrition for resistance to infection has long been appreciated but poorly understood. This has been especially true for tuberculosis. The paper by Liu and colleagues in the recent issue of *Science* (1) provides an important step toward understanding the role of vitamin D in infections by organisms such as *Mycobacterium tuberculosis*. They observed that activation of the Toll-like receptor TLR2/1 by a lipoprotein extracted from *M. tuberculosis* reduced the viability of intracellular *M. tuberculosis* in human monocytes and macrophages concomitant with increased expression of the vitamin D receptor (VDR) and of CYP27B1 (the enzyme that produces $1,25(OH)_2D_3$) in these cells. Killing of *M. tuberculosis* occurred only when the serum in which the cells were cultured contained adequate levels of 25OHD, the substrate for CYP27B1. Treatment of these cells with $1,25(OH)_2D_3$ induced the antimicrobial peptide cathelicidin, which is toxic for *M. tuberculosis*.

The paper by Liu *et al*. helps to explain a conundrum. Indeed, prior to the development of specific drugs for the treatment of tuberculosis, getting out of the city into fresh air and sunlight was the treatment of choice. In a recent survey of patients with tuberculosis in London (2), 56% had undetectable 25OHD levels, and an additional 20% had detectable levels but below 9 ng/ml (22 nM). On the other hand, hypercalcemia is commonly found in patients with tuberculosis, observed in up to 50% of such patients (3). As in sarcoidosis, the hypercalcemia is attributed to $1,25(OH)_2D_3$ production by activated macrophages in the granulomatous tissue (4). Thus we have the interesting situation in which vitamin D deficiency is associated with susceptibility to tuberculosis, but tuberculosis is associated with increased production of the active vitamin D metabolite, $1,25(OH)_2D_3$. What is going on here? The results from Liu and colleagues demonstrate that the induction of $1,25(OH)_2D_3$ production by activated monocytes and macrophages explains the hypercalcemia and increased $1,25(OH)_2D_3$ levels seen in subjects with tuberculosis. The increased vulnerability of subjects with vitamin D deficiency to tuberculosis is explained by the requirement for sufficient 25OHD as substrate for $1,25(OH)_2D_3$ production by the monocytes/macrophages to enable the induction of cathelicidin in the monocytes/macrophages to kill the intracellular bacterium.

This manuscript illustrates the importance of two non-classic aspects of the vitamin D endocrine system: the role of extrarenal production of $1,25(OH)_2D_3$ and the role of $1,25(OH)_2D_3$ outside the classic target tissues. A number of studies have established extrarenal production of

$1,25(OH)_2D_3$ in a broad range of tissues, including keratinocytes (5) from which the human CYP27B1 was first cloned (6), bone (7), chondrocytes (8), placenta (9), parathyroid gland (10), pancreas (11), colon (12), prostate (13), and breast (14), in addition to macrophages (15) as described above. The question is why do so many tissues produce $1,25(OH)_2D_3$, since the kidney is the major contributor to circulating $1,25(OH)_2D_3$? The likely answer is related to the second point, namely the role of $1,25(OH)_2D_3$ outside the classic target tissues. The receptor for $1,25(OH)_2D_3$, the VDR, is found in most tissues (16), and numerous functions for this hormone have been described. In many tissues $1,25(OH)_2D_3$ has anti-proliferative, pro-differentiating actions which have been successfully exploited in the treatment of psoriasis (17) and show promise in the treatment of cancers, including those of the prostate, breast, and colon (12-14). $1,25(OH)_2D_3$ also regulates secretion of hormones, inhibiting that of PTH (18) while promoting that of insulin (19). $1,25(OH)_2D_3$ and its analogs are widely used to prevent/treat secondary hyperparathyroidism in renal disease (18), although management of diabetes mellitus with these compounds has not received much clinical attention.

A third general action of $1,25(OH)_2D_3$, and the one most relevant to the publication by Liu *et al*. (1), is in immune regulation. Our immune defense system is both innate and adaptive. The adaptive response is slow in unimmunized individuals, and requires antigen processing and presentation by dendritic cells, with a consequent cellular immune response by T cells and antibody production by B cells. The innate system allows a rapid response, by granulocytes, monocytes, macrophages and natural killer cells, to patterns in various molecules that are conserved in infectious organisms. Macrophages express Toll-like receptors (TLRs) that recognize such patterns on infectious organisms as ligands. As is illustrated in the publication by Liu *et al*. (1), $1,25(OH)_2D_3$ plays a key role in the efficacy of the innate defense mechanism, since TLR stimulation directly leads to VDR and CYP27B1 upregulation. In contrast, much of the role of $1,25(OH)_2D_3$ in the adaptive pathway is inhibitory: inhibition of T cell proliferation (20), inhibition of the development of the Th1 T cell subset with shift to Th2 cells (21;22), decreased maturation of dendritic cells (23;24), and inhibition of IL-2 (25) and IFN-γ (25) production. These inhibitory effects on the adaptive immune response may prove clinically useful in the prevention/treatment of autoimmune diseases. The levels of $1,25(OH)_2D_3$ required for these non-classical effects, however, exceed the circulating concentrations (picomolar). Given that circulating 25OHD levels are approximately three orders of magnitude higher than those of $1,25(OH)_2D_3$, the presence of CYP27B1 in these cells may very well be critical for the ability of vitamin D (and its major circulating metabolite 25OHD) to exert local actions on the immune system. The publication by Liu *et al*. (1) illustrates this nicely in that TLR1/2-induced activation of *M. tuberculosis* killing required adequate amounts of serum 25OHD. Presumably, this enabled the macrophage to produce sufficient $1,25(OH)_2D_3$ to induce the killing mechanisms. A similar story applies for the treatment of various cancers containing CYP27B1 or for the management of diabetes mellitus and hyperparathyroidism. Raising the substrate 25OHD to enable the endogenous CYP27B1 in the cancer cell, pancreas or parathyroid gland to make the $1,25(OH)_2D_3$ locally may be more beneficial and less toxic than attempting to treat these conditions with $1,25(OH)_2D_3$ itself. The public health take-home lesson from these studies is that the circulating level of 25OHD counts, and it counts because a number of cells make their own $1,25(OH)_2D_3$, and respond to their own $1,25(OH)_2D_3$ to control important cellular functions including resistance to tuberculosis.

Conflict of Interest: The author reports that no conflict of interest exists.

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