Vitamin D₃–Triggered Antimicrobial Response—Another Pleiotropic Effect beyond Mineral and Bone Metabolism


Niels Ryberg Finsen, who received the third Nobel prize in medicine in 1903, was honored because in 1895 he had discovered a cure for a disease that had been incurable before, *i.e.*, skin tuberculosis known as lupus vulgaris (1–3). Phototherapy was performed by exposing the skin to an electrical arc lamp and producing moderate sunburn. Although alternative or complementary mechanisms have been proposed, such as generation of singlet oxygen by the porphyrin molecules in *Mycobacterium tuberculosis*, it appears that after more than 100 years the main molecular mechanism underlying Finsen’s phototherapy has been unraveled (4) and turns out to be one more of the pleiotropic effects of vitamin D₃ through genomic and nongenomic pathways (5–7).

To better understand the following, some background information is useful. If an organism is invaded by an infectious agent, the organism does rely not only on the acquired immune system (mainly antibodies and lymphocytes), but as an additional acute emergency intervention it relies also on the phylogenetically ancient innate immune system, which depends on different Toll-like receptors (TLR). Based on the recognition of specific repetitive patterns (8) in the chemical structure of the invading microorganisms’ products, *e.g.*, lipopeptides (9), the corresponding TLR is activated and triggers the synthesis of cationic antimicrobial peptides (10,11) such as cathelicidin (4) and α- or β-defensins (12). The human cathelicidin contains a C-terminal cationic, antimicrobial peptide domain that is activated by cleavage from the N-terminal cathelin portion of the propeptide, which is stored in secondary granules of neutrophils and other white cell populations. Production and secretion of cathelicidin is not restricted to myeloid cells, however. It occurs also in other cells exposed to microbes, such as the epithelial cells of the mouth, tongue, esophagus, intestine, cervix and vagina (13), lung (14), and salivary, sweat (15), and mammary glands (16).

Liu et al. (4) pursued a remarkable species difference: In mice it had been shown that the acute antimicrobial response triggered by the heterodimer TLR2/1 depends on the generation of nitric oxide (NO) (17), yet in human macrophages the antimicrobial activity of macrophages triggered by TLR 2/1 is not dependent on the generation of NO and obviously must be mediated by alternative effectors. Studies to resolve this puzzle led to an unanticipated result that extends the range of the known effects of active vitamin D₃, at least in humans—a further addition to a growing list of actions of active vitamin D₃ beyond mineral and bone metabolism.

Using intracellular *M. tuberculosis*, it had been shown (18) that activation of the heterodimer TLR1/2 reduces its viability in human monocytes and macrophages, but not in dendritic cells. Liu et al. went one step further and investigated gene expression profiles of monocytes (and of dendritic cells as controls) after exposure to a synthetic *M. tuberculosis*–specific lipopeptide acting via the heterodimer TLR2/1. In monocytes, but not in dendritic cells, the microarray and quantitative PCR techniques identified two candidates: the vitamin D₃ receptor (VDR) and the calcium-binding proinflammatory molecule S100A2. When further VDR-dependent down-stream genes were assessed, the gene coding for Cyp27B1 was upregulated (the 1α-hydroxylase catalyzing the conversion of 25(OH)D₃ to 1,25(OH)₂D₃), but not CYP24 hydroxylase (mediating the catabolism of 1,25(OH)₂D₃).

Two preceding papers (19,20) had shown that 1,25(OH)₂D₃ stimulated expression of cationic antimicrobial peptides in various cell lines. In a second step, the authors therefore tested the plausible working hypothesis that 1,25(OH)₂D₃ upregulates cationic antimicrobial peptides. They added 1,25(OH)₂D₃ to human monocytes and showed that the mRNA of the cationic...
antimicrobial peptide cathelicidin was dose-dependently upregulated. The 1,25(OH)2D3-dependent expression of active cathelicidin peptide in human monocytes was documented by flow cytometry as well as by the sophisticated SELDI-TOF (surface-enhanced laser desorption ionization–time of flight) mass spectrometry, which showed that the precursor had been processed to the active cathelicidin peptide LL-37. Increased antimycobacterial activity of 1,25(OH)2D3-treated human monocytes was documented using the techniques of 3H uracil uptake and of colony formation (CFU).

Not only 1,25(OH)2D3 but also—under certain conditions—the precursor 25(OH)D3 increased the production of cathelicidin and decreased the viability of intracellular M. tuberculosis. Activation of monocytes via the heterodimer TLR2/1 or addition of vitamin 25(OH)D3 alone were ineffective, but activation via TLR2/1 plus addition of 25(OH)D3 upregulated the antibacterial agent cathelicidin and the catabolic enzyme CYP24. Blockade of 1α-hydroxylase by the antagonist itraconazole or addition of the vitamin D3 receptor antagonist ZK 159222 abrogated production of cathelicidin and mycobactericidal activity. Addition of human serum (with higher 25(OH)D3 concentrations), but not of fetal calf serum (with lower 25(OH)D3 concentrations), reproduced the effects of exogenous 25(OH)D3.

These findings permit the conclusion that the mycobactericidal effect of activation of the heterodimer TLR2/1 depends on the following sequence:

- Availability of sufficient 25(OH)D3, induction of 1α-hydroxylase (Cyp27B1), and activation of the VDR by 1,25(OH)2D3.

Local production of 1,25(OH)2D3 explains the high local concentrations of 1,25(OH)2D3 in tuberculous lesions (21) and the frequency of hypercalcemia in nonrenal and renal patients with tuberculosis (22).

The study also provides plausible answers to two puzzling problems:

- Why is tuberculosis more frequent in blacks?
- Why does the mouse not use the above vitamin D3–dependent microbicidal pathway?

The recently observed tight correlation between skin pigmentation of different populations and presumed latitude where their ancestors lived suggests that the dark skin pigmentation in blacks (and presumably in the ancestors of us all) emerged as a defense against ultraviolet skin damage and malignancy (23). The downside is that dark-skinned blacks have lower 25(OH)D3 concentrations (24) because of their lower capacity for UV light–dependent cutaneous vitamin D3 synthesis. The authors showed that, in serum samples of blacks, the upregulation of cathelicidin mRNA via TLR2/1 activation was significantly less than that found in serum samples of whites. This may be one reason why tuberculosis is more frequent and severe in blacks (25).

Gombart et al. had shown that only primates have a consensus vitamin D3-responsive element (VDRE) in the promoter of the cathelicidin antimicrobial peptide gene, presumably as the result of a founder effect in a primate progenitor (19). Such VDRE is absent in mice, rats, and dogs. Liu et al. argued plausibly that the absence of the vitamin D3–dependent microbicidal pathway in mice is explained by the fact that they are nocturnal animals and thus are forced to use NO as the bactericidal agent, in contrast to humans, in whom, as daytime creatures, exposure to UV light permits synthesis of sufficient vitamin D3 in the skin (4).

The implications of stimulation of the synthesis of cathelicidin by vitamin D3 extend beyond the control of tuberculosis. Wang et al. (20) and Gombart et al. (19) had documented that 1,25(OH)2D3 stimulates the synthesis of cathelicidin in numerous cells other than monocytes/macrophages, such as keratinocytes, colonic cancer cells, bone marrow cells, and leukemia (AML) cells. The modulation of the synthesis of cathelicidin by 1,25(OH)2D3 extends the known role of active vitamin D3 in immunomodulation (26–29). Immunomodulatory properties have been ascribed to cathelicidin as well (19). Uremia is a microinflammatory state (30) potentially caused by, among others, bacterial triggers, e.g., dental infection (31,32) and intestinal leak (33),
penetration of lipopolysaccharide (LPS) into the circulation as a result of edema of the intestinal mucosa caused by hypervolemia (34). Cathelicidin binds to LPS and neutralizes it (35); cathelicidin also inhibits the release of TNF-α, tissue factor, and NO in response to LPS (15,35), as well as macrophage activation by LPS (29). LPS also downregulates the VDR, a potentially negative impact of the synthesis of cathelicidin (36).

Surprisingly, recent observational data has suggested that survival of dialysis patients is improved by administration of active vitamin D₃ (37,38). The potential causes are unclear. Apart from the points discussed above, possible causes include vitamin D₃ effects on the activity of systemic or local renin-angiotensin systems (39), cardiac tissue (40), and vascular tissue, which possess VDR (41) and 1α-hydroxylase (42). Whatever the explanation, active vitamin D₃ clearly is a candidate, although currently not supported by definitive evidence, for cardiovascular intervention in chronic kidney disease (43,44).

References
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