Overview

Defensin is a generic name reserved for an endogenously synthesized antimicrobial agent. The purpose of this summary is to describe a series of discoveries that led to the notion that 25-hydroxylated metabolites of vitamin D are naturally occurring, potent mediators of the human innate immune response to bacterial antigens. As such, the following discussion will: 1) highlight the basic elements of human innate immune response; 2) recount early work from our laboratory and others relevant to the extrarenal expression of the vitamin D-1-hydroxylase in the macrophage, the cellular initiator of the innate immune response; 3) review the immunobiology of the active vitamin D metabolite, 1,25-dihydroxyvitamin D (1,25-D); and 4) describe the relevance of the vitamin D intracrine-autocrine-paracrine system in a model of a common and devastating human disease, tuberculosis.

Toll-like receptors and the innate immune response

The initiating step in the mammalian innate immune response is a breech in the epithelial barrier between the bacterial-laden outside environment and the relatively sterile internal environment of the host. Once the bacterial products, so-called pathogen-associated molecular patterns or PAMPs, gain access to the host interior, they are recognized by a subclass of pattern recognition receptors embedded in the plasma membrane of macrophages. These are the toll-like receptors (TLRs). There are 11 known receptors and an array of widely varying kinds of antigens, including peptides, lipids and nucleic acids. The liganded receptors recruit MyD88 adaptor proteins, which in turn, trip a number of intracellular signalling pathways, many of which terminate in the transaction of NFκB. The end result is antigen destruction, initiation of the T- and B-cell adaptive immune response and, in some cases, tissue injury.

Vitamin D and the human immune response

Data from our lab and many others promote the concept that the active vitamin D hormone, 1,25-D, stimulates the innate immune response in antigen-presenting cells, like macrophages and dendritic cells, while at the same time acts to squelch any overzealous responsivity in the adaptive immune response to the offending antigen. For example, when activated non-specifically with mitogen or specifically with antigen, such as the tuberculous bacillus, the macrophage becomes a target for 1,25-D by expressing the vitamin D receptor (VDR). Once expressed, the VDR interacts with the macrophage, mitogen or antigen-activated lymphocytes also express the VDR, all designed to rid the host of the offending agent. Like the macrophage, mitogen or antigen-activated lymphocytes also express the VDR. However, completely unlike the macrophage, the 1,25-VDR interaction in the lymphocyte leads to inhibition of T-cell differentiation and proliferation, Th1 cell immunoactivity and interleukin 2-driven B-cell immunoglobulin production.

The key point being that the 1,25-D in play here did not originate from the general circulation. Rather, the hormone was generated locally to act locally on both macrophages and lymphocytes in the inflammatory microenvironment. This led to the postulate that this locally-produced 1,25-D was evidence of a primitive, non-endocrine biological system designed to control immune responsiveness to invading antigen; a system in existence in vertebrate species long before it morphed into an endocrine system to control calcium and phosphate homeostasis. Generally speaking these local immunoregulatory effects of the active vitamin D metabolite are below the limit of our detection clinically; it is not until sufficient amounts of 1,25-D escape the confines of the local inflammatory microenvironment into the serum do we, as
clinicians, begin to see the hormonal effects (i.e., hypercalciuria or frank hypercalcemia) of this cytokine.

It is now known that it is the macrophage that is the source of 1,25-D in the local inflammatory microenvironment, and that this 1,25-D is the synthetic product of the CYP27B1-hydroxylase gene. However, compared to the CYP27B1 as it is expressed at the endocrine source of the hormone in the proximal renal tubular epithelial cell (see Table 1), in the macrophage the 1-hydroxylase is not accompanied by a functional vitamin D-24-hydroxylase. As such, the CYP27B1 gene product in the macrophage is 25-D substrate dependent, explaining why the enzyme is immune to feedback inhibition by 1,25-D and why patients with an extrarenal, macrophage source for 1,25-D encounter hypercalcemic and hypercalciuric problems more frequently in the summertime when 25-D levels are at their seasonal peaks. Also dissimilar from the renal CYP27B1, the macrophage 1-hydroxylase is immune to stimulation by parathyroid hormone (PTH); macrophages are devoid of the PTH-PTHrP receptor. By contrast, the macrophage CYP27B1 is stimulated by the TLR4 ligand LPS and the tuberculosis TLR2/1 ligand as well as by the monokine IFNγamma. The activity of the enzyme is associated with the inflammatory index of the underlying disease and driven by endogenously-synthesized nitric oxide (NO).

**Vitamin D and tuberculosis**

The pleural effusion fluid of patients with active tuberculosis is known to be enriched in macrophages, which secrete substantial amounts of 1,25-D under the influence of IFNγamma. As summarized in Figure 1, recent work now demonstrates that interaction of the TLR2/1 dimer pair in the macrophage membrane triggers upregulation of expression of both the VDR and CYP27B1 genes with pathogen-associated molecular pattern molecules or PAMPs shed from the cell wall *Mycobacterium tuberculosis* (M.t.b.). This permits the macrophage to make use of serum vitamin D binding protein (DBP)-bound 25-hydroxyvitamin D (25-D) in the extracellular fluid to be internalized and used as substrate for the upregulated CYP27B1 and endogenous synthesis of 1,25-D. The locally produced 1,25-D is then free to interact with the upregulated VDR gene product, engage the retinoid X receptor and transactivate the cathelicidin gene. In other words, the M.t.b. PAMP ligand-TLR interaction initiates a series of vitamin D-dependent cellular events that culminate in the ingestion and killing of the invading mycobacterium under the influence of the defensin-like molecule cathelicidin.

These data predict that a decrement in the extracellular

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**Table 1.** Functional characteristics of the macrophage vitamin D-1-hydroxylase distinct from the renal 1-hydroxylase.

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<tr>
<th>Characteristic</th>
<th>Macrophage</th>
<th>Renal Source</th>
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<tbody>
<tr>
<td>Substrate dependent</td>
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<td>Immune to 1,25-D inhibition</td>
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<td>Immune to PTH stimulation</td>
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<td>Stimulated by LPS</td>
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<td>Stimulated by PPD (TB)</td>
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<td>Stimulated by IFN</td>
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<td>Correlated with disease activity</td>
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<td>Driven by endogenous NO</td>
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**Figure 1.** Vitamin D-directed antimicrobial effects in human macrophages.
content of 25-D may be a limiting factor for effective M.tb killing. To test this hypothesis Liu and colleagues examined human monocyte-macrophage cathelicidin expression and mycobacterial killing in cells incubated with vitamin D (25-D)-deficient serum from sunlight-deprived African Americans and from a group of vitamin D-sufficient Caucasians. The 25-D-insufficient serum was much less capable of supporting cathelicidin gene expression and bacterial killing than was vitamin D-sufficient serum. However, this significantly diminished cathelicidin and antimicrobial effect of vitamin D-deficient serum on macrophage function could be rescued by restoring the extracellular 25-D levels to normal.

A clinical trial is currently underway trying to replicate this rescue experiment in vitro using paired sera from human subjects pre- and post-repair of vitamin D insufficiency in vivo.

References