The Epidermal Vitamin D System and Innate Immunity: Some More Light Shed on This Unique Photoendocrine System?

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The Epidermal Vitamin D System

The skin occupies a central position within the vitamin D system. The epidermis is the main site of vitamin D₃ photosynthesis out of 7-dehydrocholesterol (provitamin D₃) [1, 2], the last precursor in the cholesterol synthesis [3]. Epidermal keratinocytes express the vitamin D hydroxylase enzymes 25-hydroxylase (CYP27A1) and 1 α -hydroxylase (CYP27B1), enabling them to convert vitamin D₃ into 25-hydroxyvitamin D₃ (25OHD) and 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] (also known as calcitriol), the biologically active form of vitamin D₃ [4]. In addition, keratinocytes are vitamin D target cells as they contain the vitamin D receptor (VDR) and respond to 1,25(OH)₂D₃ with changes in proliferation, differentiation and cytokine production [5]. Taken together, these findings indicate the existence of a unique photoendocrine vitamin D system in keratinocytes which is corroborated by the demonstration of 1,25(OH)₂D₃ synthesis and vitamin D effects in UVB-irradiated skin or keratinocytes [3, 4]. In contrast to epidermal keratinocytes, dermal fibroblasts only have the capacity for photoproduction of 25OHD but not 1,25(OH)₂D₃ [6]. 25OHD may then act as a paracrine factor to keratinocytes. Monocytes, like keratinocytes, have the full machinery to photoproduce 1,25(OH)₂D₃ (fig. 1) [7].

The importance of vitamin D in cutaneous biology and pathology is further underlined by the skin phenotype (alopecia) in mice or humans with a dysfunctional

VDR (by disruption of ligand-independent VDR effects on keratinocyte stem cell function and hair cycling) [13] and by the successful use of vitamin D analogues in psoriasis [14, 15].

Yet the physiologic significance of the cutaneous photosynthesis of 1,25(OH)₂D₃ is still poorly understood. As the amounts of UVB-produced 1,25(OH)₂D₃ in keratinocytes are very small [3] and circulating 25OHD and 1,25(OH)₂D₃ are hardly detectable in hepatectomized or nephrectomized animals [16], cutaneous production of active vitamin D does not appear to play a major role outside the skin. However, it is tempting to speculate that locally produced 1,25(OH)₂D₃ may contribute to UVB effects within the skin, such as its therapeutic action on psoriasis [1]. Furthermore, photoproduced 1,25(OH)₂D₃ might serve as an endogenous protection mechanism against UVB-dependent DNA damage, apoptosis and release of proinflammatory cytokines such as IL-6 [17, 18].

Vitamin D and Innate Immunity: Of Light and Infection – Finsen Revisited?

More than 25 years have passed since the first evidence of a direct link between vitamin D and innate immunity by the demonstration that calcitriol induces monocyte differentiation [19, 20]. In addition to phagocytes and cytokines, a major component of the innate immune system is a diverse combination of cationic pep-

Fig. 1. The epidermal vitamin D system and innate immunity. Epidermal keratinocytes [3] as well as monocytes [7] contain the full machinery for UVB-dependent photosynthesis of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] from 7-dehydrocholesterol (7-DHC). Photoproduced vitamin D₃ is further hydroxylated to 25-hydroxyvitamin D₃ (25OHD) by 25-hydroxylase (CYP27A1) and subsequently to 1,25(OH)₂D₃ by 1 α -hydroxylase (CYP27B1). Toll-like receptor (TLR)-2 activation by microbial extracts induces CYP27B1 expression in keratinocytes [8]. Fibroblasts can photoproduce 25OHD – possibly acting as a paracrine factor for monocytes or keratinocytes – but not 1,25(OH)₂D₃ [6]. When 1,25(OH)₂D₃ is produced in sufficient amounts, it activates the vitamin D receptor to induce transcription of the TLR-2 gene in keratinocytes [8] and of the cathelicidin gene in monocytes [9] and keratinocytes [10]. Cathelicidin-derived peptides not only exert antimicrobial activity [9] but also influence adaptive immunity, angiogenesis and cell proliferation [11]. Therefore, cathelicidin appears to be involved in wound healing [8] and inflammatory skin diseases such as rosacea [12] and psoriasis [11].

tides that include the α - and β -defensins and cathelicidins, which have potent microbicidal activities at low concentrations [11]. The recent identification of the antimicrobial peptide cathelicidin as a vitamin D target gene [21], and of CYP27B1 and VDR upregulation in monocytes as the link between tuberculosis Toll-like receptor-2 (TLR-2) activation on the one hand and cathelicidin production and intracellular mycobacteria killing on the other hand [9], added an entirely new dimension to the vitamin D system. Increased CYP27B1 expression and 1 α -hydroxylase activity thus account for increased monocyte 1,25(OH)₂D₃ production acting through higher numbers of VDR. As this mechanism relies on adequate circulating 25OHD levels (main serum vitamin D metabolite), vitamin D status becomes directly linked to in-

nate immunity. Therefore, it may explain the increased susceptibility of African-American individuals to tuberculosis [9] and the seasonal peaking of viral infections (influenza) in winter [22].

Cathelicidin expression was also shown to be increased in human skin in vivo by topical application of active vitamin D compounds such as calcipotriol and 1,25(OH)₂D₃ [10]. Moreover, UVB irradiation led to elevated cutaneous cathelicidin mRNA expression in vivo paralleled by an increase in VDR mRNA [23]. UVB-dependent production of 1,25(OH)₂D₃ in the skin with subsequent induction of cathelicidin (fig. 1) is therefore thought by some to underlie the therapeutic effect of phototherapy in lupus vulgaris, for which Finsen received the Nobel prize more than a century ago [24]. However, the lamps

Finsen used for his treatment were recently investigated and found not to emit within the UVB spectrum [25]. It was concluded that a photodynamic effect of a *Mycobacterium tuberculosis* porphyrin was the likely basis for Finsen's successful treatment of skin tuberculosis [25]. But how to reconcile this hypothesis with the painlessness of Finsen's phototherapy [25]? Another question is whether UVB irradiation increases the epidermal concentrations of $1,25(\text{OH})_2\text{D}_3$ sufficiently to induce cathelicidin: indeed, UVB irradiation of keratinocytes generates physiological rather than pharmacological concentrations of $1,25(\text{OH})_2\text{D}_3$ [3] that are unlikely to induce cathelicidin. Moreover, induction of cathelicidin in UVB-irradiated human skin [23] was not proven to be vitamin-D-dependent. Increased photosynthesis of vitamin D in the skin that replenishes the serum with 25OHD after hydroxylation in the liver might therefore be a more plausible explanation for Finsen's phototherapy [9, 26], if it was to contain UVB at all. The same argument also pleads against the involvement of locally produced active vitamin D in the effectiveness of UVB for psoriasis [1] and as an endogenous photoprotective pathway [17, 18].

In contrast to humans and primates, cathelicidin expression is not vitamin-D-regulated in rodents [27]. This is explained by their nocturnal life, which precluded evolution from taking benefit from the biological effects of sunlight [11]. Interestingly, in cats (other nocturnal animals), cutaneous 7-dehydrocholesterol Δ^7 -reductase activity (converting 7-dehydrocholesterol into cholesterol) was shown to be so high that the 7-dehydrocholesterol levels in the skin were insufficient to allow vitamin D_3 photosynthesis [28]. Therefore, cats depend on their diet for their vitamin D supply (making vitamin D a true vitamin in these animals). That the ability for cutaneous photoproduction of vitamin D seemingly paralleled the evolution of cathelicidin to vitamin D regulation might suggest a link between these 2 events.

Vitamin D and Cathelicidin: Role in Wound Healing and Inflammatory Skin Disease

Cathelicidin is not only an effector molecule of innate immunity by its antimicrobial activity but it also exhibits biological activities on adaptive immunity, angiogenesis as well as cell proliferation and migration [11]. Therefore, its function is pivotal for wound repair [8]. In keratinocytes, CYP27B1 expression is induced by microbial stimulation of TLR-2, like in monocytes, and by the cytokine transforming growth factor β [8]. In the setting of wound

healing, this activation of TLR-2 resulted in $1,25(\text{OH})_2\text{D}_3$ biosynthesis as indirectly shown by strong induction of 24-hydroxylase [the most vitamin-D-responsive gene known encoding the first enzyme in the catabolism of $1,25(\text{OH})_2\text{D}_3$] [8]. Moreover, vitamin-D-dependent cathelicidin appears to play a protagonist role in wound healing as demonstrated by 2 positive feedback loops, namely induction of TLR-2 [8] and transforming growth factor β [5] through $1,25(\text{OH})_2\text{D}_3$. These findings definitely warrant further research into the use of vitamin D (analogues) for compromised wound healing (where lower cathelicidin levels have been observed) [11]. In this respect, it is also a burning question whether UVB phototherapy is capable of sufficiently stimulating cutaneous $1,25(\text{OH})_2\text{D}_3$ synthesis or cathelicidin expression to exert therapeutic effects in chronic wounds. Unfortunately, VDR expression was not looked at in this wound healing study yet [8]. As the keratinocyte VDR levels are extensively regulated by cell adhesion, cytoskeletal integrity, growth factors, cytokines, differentiation and UVB [29–32], regulation by TLR-2 can be expected as well.

In addition to its upregulation in the skin following cutaneous injury, high expression of cathelicidin and of antimicrobial peptides has also been noted in psoriasis, accounting for the rare occurrence of skin infections in this condition [11]. The effectiveness of vitamin D analogues in psoriasis [14, 15] suggests, however, that cathelicidin is not an insurmountable pharmacological target in this disease.

In contrast, decreased antimicrobial peptide expression is seen in atopic dermatitis, chronic leg ulcers and thermal burns [11, 33]. However, vitamin D analogues are not effective for atopic eczema, indicating that pharmacologic induction of cathelicidin by vitamin D is not an adequate therapeutic strategy at first sight. Induction of thymic stromal lymphopoietin (a key cytokine in the pathogenesis of atopic dermatitis) in keratinocytes by active vitamin D [34] may account for these unfavorable effects of vitamin D analogues in eczema.

Some thoughts can also be made on azole antimycotics such as ketoconazole and itraconazole, which are widely used in dermatology as topical or oral antifungal agents but also block the cytochrome P450-dependent 1α -hydroxylase and thus $1,25(\text{OH})_2\text{D}_3$ production in the skin and kidney [3, 11, 35]. It is unknown whether these agents interfere with the generation of antimicrobial peptides in treated patients and hence may alter the innate immune system or compromise wound healing. Moreover, inhibition of cathelicidin expression by topical ketoconazole might account for its anti-inflammatory ef-

fects in seborrheic dermatitis. Finally, given the recently acknowledged involvement of cathelicidin in the pathophysiology of rosacea [12], the mechanism of action of metronidazole (known to inhibit some cytochrome P450 enzymes) might be reassessed in a similar way as well as the role of sunlight as a rosacea-provoking factor.

Conclusion

Taken together, the link between altered vitamin D metabolism as an intermediary for an important pathway of innate immunity (induction of antimicrobial peptides

by TLR activation) opens exciting new horizons for vitamin D research in general and the epidermal photoendocrine vitamin D system in particular, not only for the study of anti-infectious immune defense mechanisms but also for a better understanding and treatment of inflammatory skin diseases or compromised wound healing. Whether cutaneous photosynthesis of 1,25(OH)₂D₃ directly enhances innate immunity in the skin or whether skin-photoproduced vitamin D₃ acts via hepatic conversion to 25OHD to reach target cells including monocytes and keratinocytes remains to be determined. Obviously some more (UVB) light needs to be shed on this issue.

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