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VITAMIN D, SUNLIGHT AND OUR SKIN

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SUMMARY

This paper reviews historical, clinical, animal and molecular-based studies linking sunlight, vitamin D and the immune system to maintaining the barrier defence and health of the skin. It focuses on the discovery that vitamin D regulates the expression of antimicrobial peptides important in skin immunity with an emphasis on the cathelicidin antimicrobial peptide. The dysregulation of antimicrobial peptide expression, altered composition of microbiota in skin and use of UVB heliotherapy and vitamin D are discussed. Findings from recent and past studies indicate that vitamin D mediated regulation of the cathelicidin gene may provide approaches for therapy of skin infections and disorders. Also, there is emerging evidence that cutaneous synthesis of vitamin D and induction of the cathelicidin antimicrobial peptide gene may provide a mechanism by which sunlight and vitamin D may modulate the skin microbiota.

INTRODUCTION

Historically the ancient Egyptians, Babylonians, Assyrians, Greeks and Romans recognized the health benefits of the sun. Sunbathing to restore health or heliotherapy was promoted by Hippocrates and extoled by Herodotus. Pliny the Elder remarked "Sol est remediorum maximum" or the "sun is the best remedy" (*Levine*, 1971). Sunlight effectively treated rickets and in the 19th and 20th centuries and heliotherapy at sanatoriums was standard treatment for tuberculosis (*Howson*, 1928; *Koch*, 1901; *Rajakumar,* 2003). Niels Ryberg Finsen won the 1903 Nobel Prize in Medicine and Physiology for his discovery that heliotherapy with artificial sun light effectively treated lupus vulgaris a cutaneous infection with *Mycobacterium tuberculosis* (*Mtb*) (*Grzybowski* and *Pietrzak*, 2012). It wasn't until the 1920s that vitamin D, a major curative component created by the sun, was identified through a series of elegant studies by Adolf Windaus, Harry Goldblatt, Harry Steenbock, Alfred Hess and Mildred Weinstock (*Norman*, 2012). Hess and Weinstock verified light produced vitamin D by demonstrating that ultraviolet light irradiation of small portions of skin from rachitic rats cured other groups of rachitic rats fed the skin. This was not observed with non-irradiated skin (*Norman*, 2012). These discoveries led to the fortification of foods with vitamin D and the eradication of rickets in the United States and the development of a high-dose oral therapy with vitamin D for lupus vulgaris in the 1940s (*Dowling*, 1946; *Gaumond*, 1948). Furthermore, these discoveries identified vitamin D as a likely explanation for the healing properties of the sun.

THE VITAMIN D PATHWAY

A vitamin is required for normal physiological processes, is not synthesized by the body and must be acquired regularly from the diet. By this definition vitamin D is a misnomer as the diet is a poor source and the most effective way to acquire vitamin D is through synthesis in the skin or consumption of a purified supplement. Briefly, natural sunlight or artificial ultraviolet B rays cleave the B-ring of 7-dehydrocholesterol in the skin to produce cholecalciferol or vitamin D_3. Vitamin D_3 is absorbed into the blood and circulates to the liver where it is hydroxylated by the cytochrome p450 enzyme CYP27A1 to calcidiol or 25-hydroxyvitamin D_3 [$25(OH)D_3$]. This form circulates in the blood and is measured in the serum as an indicator of vitamin D status. Calcidiol is converted to its bioactive form, calcitriol or 1α,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], by the mitochondrial 1α-hydroxylase enzyme CYP27B1 in the kidney. $1,25(OH)_2D_3$ binds to a transcription factor called the vitamin D receptor (VDR). This steroid-hormone nuclear receptor binds to specific sites in the genome and interacts with cofactors to activate and/or repress the expression of target genes (*Mangelsdorf* et al., 1995; *Christakos* et al., 1996).

The renal synthesis of calcitriol is essential for efficient uptake of dietary calcium for bone health. A drop in circulating Ca^{2+} levels stimulates the production of parathyroid hormone (PTH) that, in turn, induces CYP27B1 expression by primary renal tubules. This increases $1,25(OH)_2D_3$ production, which activates Ca^{2+} transporter expression through the VDR in the small intestine. The increase in circulating Ca^{2+}, suppresses PTH production. In a negative feedback loop, activated VDR binds to the *CYP27B1* promoter and represses its expression. Also, VDR induces fibroblast growth factor-23 in osteocytes which inhibits secretion of PTH and inhibits CYP27B1 and stimulates CYP24A1 a mitochondrial enzyme that catabolizes both $1,25(OH)_2D_3$ and $25(OH)\ D_3$ to limit $1,25(OH)_2D_3$ levels and prevent hypercalcaemia (*Zierold* et al., 1995; *Saito* et al., 2003; *Paz* et al., 2007).

An abundance of epidemiological, clinical and basic research has highlighted the potential roles of vitamin D in preventing cancer, autoimmune disorders, cardiovascular disease and infections (*Grober* et al., 2013). The synthesis of calcitriol in non-renal tissues and cells is likely important for mediating these additional health benefits (*Hewison* et al., 2004). The extra-renal synthesis of $1,25(OH)_2D_3$ occurs in lung, colon, parathyroid glands, bone, skin and macrophages. In immune cells it is considered important for optimal immune response at sites of infection (*Hewison* et al., 2004).

VITAMIN D AND IMMUNITY

The historical connection between sources of vitamin D and successful treatment of tuberculosis highlighted an important early link with immune function. The connection of vitamin D to immunity was strengthened by the discovery that the VDR is expressed in T and B cells, monocytes, macrophages, dendritic cells (DCs) and neutrophils (*Bhalla* et al., 1983; *Provvedini* et al., 1983; *Mangelsdorf* et al., 1984; *Brennan* et al., 1987; *Kreutz* et al.,

1993; *Deluca* and *Cantorna,* 2001; *Takahashi* et al., 2002; *Adorini* et al., 2004;). $1,25(OH)_2D_3$ inhibits Th17 development, increases the *frequency* of Th2 and regulatory T-cells, decreases Th1 development, and modulates T-cell proliferation and cytokine expression (*Lemire* et al., 1995; *Penna* and *Adorini,* 2000; *Boonstra* et al., 2001; *Daniel* et al., 2008). $1,25(OH)_2D_3$ also promotes tolerance in dendritic cells and T-cells and inhibits B-cell differentiation into plasma cells (*Mathieu and Adorini,* 2002; *Adorini* et al., 2004; *Chen* et al., 2007). Overall vitamin D appears to mediate an anti-inflammatory response and promote tolerance in the adaptive response.

In addition to responding to circulating $1,25(OH)_2D_3$, dendritic cells, macrophages and T-cells actively metabolize $1,25(OH)_2D_3$ (*Hewison,* 2012). Extra-renal production of $1,25(OH)_2D_3$ by macrophages from some granulomatous disease patients was reported (*Barbour* et al., 1981; *Adams* et al., 1983). *In vitro* studies with non-disease macrophages suggested that CYP27B1 activity was induced as part of the normal immune response (*Koeffler* et al., 1985; *Reichel; Koeffler* and *Norman,* 1986). DCs confer specific homing properties upon T cells during the adaptive immune response. Sigmundsdottir and colleagues demonstrated the importance of local production of active vitamin D on T-cell homing to the skin. DCs derived from the skin were able to synthesize $1,25(OH)_2D_3$ from vitamin D_3. This induced expression of CC chemokine receptor 10 in T cells and suppressed expression of gut-homing receptors, which enabled T cells to migrate toward the chemokine CCL27 that is secreted by keratinocytes in the epidermis. Their findings support a model that DCs utilize locally produced vitamin D to program T-cell epidermal tropism (*Sigmundsdottir* et al., 2007).

The production of potentially high local levels of $1,25(OH)_2D_3$ could be important for intracrine and paracrine pathways that may influence the interactions between vitamin D, the immune system and pathogens (*Hewison,* 2012). During the mid-1980s, a link between vitamin D-deficiency and impaired immune defence to *Mtb* was proposed and it was demonstrated that both $25(OH)D_3$ and $1,25(OH)_2D_3$ increased the ability of human monocytes to control *Mtb* proliferation (*Davies,* 1985; *Rook* et al., 1986). Nearly 20 years later the mechanism for this increased killing by human monocytes/macrophages was elucidated. Several groups discovered that vitamin D increased expression of the cathelicidin antimicrobial peptide (*CAMP*) gene (*Wang* et al., 2004; *Gombart; Borregaard and Koeffler,* 2005; *Weber* et al., 2005). This vitamin D-mediated induction was subsequently shown to occur through activation of Toll-like receptor 2/1-signalling using the synthetic 19-KD *Mtb*-derived lipopeptide (*Liu* et al., 2006). Activation of macrophages induced CYP27B1 and VDR expression and increased conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$. The increase in ligand activated VDR, in turn, induced CAMP. Liu and colleagues further showed in an *in vitro* assay that insufficient levels of serum $25(OH)D_3$ resulted in a lack of CAMP induction upon activation of macrophages, consistent with the observation that vitamin D-deficiency correlates with increased susceptibility to *Mtb* infection (*Liu* et al., 2006).

The human ß-defensin 2 or *DEFB4* gene was also identified as a vitamin D inducible antimicrobial peptide gene, but its induction by vitamin D or TLR2/1 activation was not as dramatic or robust as that of CAMP (*Wang* et al., 2004; *Liu* et al., 2006). Subsequent

work showed that co-treatment of monocytes with IL-1 and $1,25(OH)_2D_3$ induced *DEFB4*. This induction was concomitant with binding of both NF-κB and VDR to the *DEFB4* promoter (*Liu* et al., 2009). In the absence of $1,25(OH)_2D_3$, the intracellular pattern recognition receptor nucleotide-binding oligomerization domain protein 2 (NOD2) by muramyl dipeptide (MDP) activates NF-κB and there is a modest induction of the *DEFB4* gene (*Voss* et al., 2006; *Wang* et al., 2010); however, pre-treatment with $1,25(OH)_2D_3$ followed by MDP leads to a robust, synergistic induction of the *DEFB4* gene (Fig. 1B) (*Wang* et al., 2010). The MDP signal was amplified because $1,25(OH)_2D_3$ strongly induced expression of NOD2 in primary human monocytic and epithelial cells (*Wang* et al., 2010). Taken together, activation of the vitamin D pathway alone is not sufficient to induce robust expression of *DEFB4* and additional signalling pathways are required (*Liu* et al., 2009; *Wang* et al., 2010). It was further demonstrated that knockdown of either DEFB4 or CAMP expression decreased killing of *Mtb* by macrophages indicating their importance to fighting the infection (*Liu* et al., 2009). An important mechanism for killing *Mtb* appears to be the ability of vitamin D to induce autophagy and the requirement of CAMP induction to promote this process (*Hoyer-Hansen* et al., 2005; *Wang* et al., 2008; *Yuk* et al., 2009). Additional findings support a paracrine macrophage-lung epithelial cell signalling pathway that is driven by IL-1β and $1,25(OH)_2D_3$ (*Verway* et al., 2013). In this model, $1,25(OH)_2D_3$ increased IL-1β secretion in *Mtb*-infected macrophages. The secreted IL-1β induced *DEFB4* expression from airway epithelial cells, which enhanced control of *Mtb* growth in co-cultured macrophages *in vitro* (*Verway* et al., 2013). Taken together these studies support an important role for vitamin D in modulating the immune response to infection.

SKIN, VITAMIN D, ANTIMICROBIAL PEPTIDES AND BARRIER DEFENCE

The epidermis is comprised of four layers of keratinocytes at different stages of differentiation: the stratum basale (basal layer), stratum spinosum (spinous layer), stratum ganulosum (granular layer) and the stratum corneum (cornified layer). The basal layer contains stem cells that proliferate and differentiate into the cells of the upper layers. As the cells leave the basal layer they express keratins, involucrin and transglutaminase K in the spinous layer. Above this, the granular layer contains keratohyalin granules that contain profilaggrin, loricrin and lamellar bodies which dump their contents composed of lipids and antimicrobial peptides into the extracellular space between the granular and cornified layers to generate the permeability barrier of the skin (*Muehleisen* et al., 2012). The cornified layer is comprised of cells that form an impermeable outer layer of dead cells that creates a barrier to invasion. Disruption of the barrier triggers induction of antimicrobial peptides and CAMP is critical for protecting the skin against infection as demonstrated by enhanced susceptibility of the *Camp* knockout mouse to Group A *Streptococcus* (*Nizet* et al., 2001). The activation of the vitamin D pathway through TLR-signalling has been described in epithelial keratinocytes. Fol-

lowing wounding of the skin, the expression of TGF-β induces CYP27B1 to produce $1,25(OH)_2D_3$ and activate intracrine expression of CAMP and TLR2 to combat infections that could occur with epidermal injury (*Schauber* et al., 2007). TGF-β and $1,25(OH)_2D_3$ also induce expression of 5-lipo-oxygenase which catalyses the synthesis of leukotrienes in monocytes (*Harle* et al., 1998). Leukotrienes are involved in leukocyte chemo-attraction at sites of infection and phagocytosis of bacteria and trigger processing of hCAP18 to LL-37 by neutrophils (*Peters-Golden* et al., 2005; *Wan* et al., 2007). In addition, to directly killing pathogens, LL-37 recruits immune cells and contributes to neo-angiogenesis and wound healing (*Koczulla* et al., 2003; *Heilborn* et al., 2003).

The peptide LL-37 is the predominant form found in neutrophils, but it is a minor constituent of the forms found in and on the human skin and surface (*Yamasaki* et al., 2006). Processing of hCAP18 in the granular layer by kallikreins, both of which are stored in lamellar granules, allows release of the peptides into the cornified layer (*Yamasaki* et al., 2006). Another source of cathelicidin on the skin's surface is secretion of LL-37 from the eccrine gland in sweat and sebocytes (*Murakami* et al., 2002; *Lee* et al., 2008). The composition of the peptides may impact the antimicrobial versus host cell signalling properties of LL-37. The shorter peptides have more potent antimicrobial properties whereas the longer LL-37 form has more potent inflammatory properties through stimulating host immune cell receptors and inducing chemokine release and immune chemo-attraction (*Braff* et al., 2005). These "alarming" properties provide an additional mechanism by which vitamin D could modulate immune response (*Yang* and *Oppenheim,* 2004). Dysregulation of cathelicidin expression and processing has been implicated in atopic dermatitis, psoriasis and rosacea (*Lande* et al., 2007; *Yamasaki* et al., 2011; *Kopfnagel* et al., 2013). In psoriasis, the overexpression of LL-37 increases TLR9 activation in plasmacytoid DCs (pDCs) contributing to the development of disease. Paradoxically, an effective treatment is topical administration of vitamin D analogues which can induce *CAMP* gene expression in healthy skin (*Weber* et al., 2005). It has not been determined if topical vitamin D affects LL37 levels in psoriasis patients, but vitamin D does interfere with the capacity of pDCs to induce T cell proliferation and secretion of INF-γ, thus limiting inflammation (*Karthaus* et al., 2014).

The epidermis is the key source of vitamin D for the body and keratinocytes possess all of the machinery (enzymes CYP27A1 and CYP27B1) to metabolize vitamin D to its bioactive form, $1,25(OH)_2D_3$ (*Muehleisen* et al., 2012). Keratinocytes express the VDR and $1,25(OH)_2D_3$ regulates the proliferation and sequential differentiation of keratinocytes into the cells that form the upper epidermal layers (*Muehleisen* et al., 2012). Loss of CYP27B1 or VDR leads to hyper-proliferation of the basal layer and defects in differentiation of the upper layers, which ultimately abrogates permeability barrier formation and the immune response (*Panda* et al., 2001; *Dardenne* et al., 2001; *Xie* et al., 2002). The skin is capable of epidermal synthesis of $1,25(OH)_2D_3$ upon exposure to UVB rays (*Lehmann* et al., 2003). This could be biologically important for regulation of cell proliferation, differentiation and immunity in the skin. Indeed, Mallbris and colleagues demonstrated that exposure to a single dose of UVB, but not UVA significantly upregulated CAMP expression in the skin of eight healthy

fair-skinned volunteers (*Mallbris* et al., 2005). The induction of vitamin D target genes in the skin by UVB exposure could affect the immune status and barrier function of the skin. It also raises the question, what is the impact on the composition of the microbiota?

SUNLIGHT, VITAMIN D, CATHELICIDIN AND MICROBIOTA

Microbiota studies are most advanced with respect to the gut. The gut is home to roughly 10^{14} bacteria comprised of 500-1,000 distinct species (*Gill* et al., 2006). This complex community is critical for host metabolism, barrier function and crosstalk with the immune system (*Hooper* et al., 2002; *Turnbaugh* et al., 2006; *Hooper and Macpherson,* 2010). Intestinal homeostasis is essential for health of the host as disruption of this balance, or dysbiosis, is associated with chronic intestinal inflammation, disorders of the gut, obesity, metabolic syndrome (MetS) and impaired barrier defence and immunity (*Sartor,* 2008; *DuPont* and *DuPont,* 2011; *Tilg* and *Kaser,* 2011; *Tehrani* et al., 2012). Maintenance of gut homeostasis depends on adequate epithelial barrier defence that is affected by interactions between the immune system, microbiota and diet. The host innate immune system is a key component in maintaining a "good fence" between the host and its microbiota and knocking-out specific genes in mice has highlighted the role of innate immunity in determining the composition of these intestinal "neighbours" (*Salzman* et al., 2010; *Vijay-Kumar* et al., 2010; *Caricilli* et al., 2011; *Kellermayer* et al., 2011). Activation of TLRs promotes epithelial cell proliferation and secretion of IgA and AMPs into the gut lumen (*Abreu,* 2010). Mice lacking TLR-5 or TLR-2 develop many features of metabolic syndrome and this correlates with altered composition of the gut microbiota and transplantation of these microbiota into wild-type germ-free mice confers many of the hallmarks of metabolic syndrome to the recipients (*Vijay-Kumar* et al., 2010; *Caricilli* et al., 2011; *Kellermayer* et al., 2011). Nucleotide-binding, oligomerization domain 2 (NOD2)-deficiency leads to altered composition of the host microbiota, both in mice and in humans, which may explain NOD2's

(*Rehman* et al., 2011). TLR and NOD2 deficiency impacts a multitude of signalling pathways in the cell and there is a large gap in our knowledge of the molecular mechanisms downstream of TLRs that regulate the composition of the microbiota; Because AMPs directly kill microorganisms, they are ideal mediators to consider. Salzman and colleagues demonstrated that deficiency of Paneth cell defensins or expression of a human-specific Paneth cell defensin (α-defensin 5, *DEFA5*) in mice resulted in significant defensin-dependent changes in the composition of the microbiota (*Salzman* et al., 2010). There were significant losses in segmented filamentous bacteria in DEFA5-expressing mice and a reduction in interleukin-17 (IL-17)-producing lamina propria T-cells (*Salzman* et al., 2010). The role of CAMP in modulating the gut microbiota is not known, but we have compared the metabolomic profile of faecal material from wild type and CAMP knockout mice and identified differences in metabolism in bile acids, phospholipids and amino acids that involve gut microflora. These preliminary data suggest that loss of CAMP may affect the com-

position of the microbiota and thus, gut metabolism (our unpublished observations).

Like the gut, the skin has a thriving microbial ecosystem of diverse ecological niches from moist to dry areas, sebaceous areas and areas with varied densities of hair, skin folds and thicknesses. This varied geography leads to differences in microbiota composition in the different body regions (*Hannigan* and *Grice,* 2013). Changes in microbiota composition are associated with these diseases and may contribute to the disease condition. *Staphylococcus aureus* colonization of the skin is associated with atopic dermatitis and treatment with antimicrobials decreases severity, but a microbial cause is not clear (*Huang* et al., 2009). It has been observed that a decreased bacterial diversity correlates with disease severity (*Kong* et al., 2012). In psoriasis, decreased representation of *Propionibacterium* and increased representation of Firmicutes in plaques is observed when compared to normal or uninvolved skin (*Fahlen* et al., 2012). The connection between aberrant AMP expression, shifts in the microbiota composition and disease pathology remains to be elucidated.

Altered host immune function is associated with changes in the skin microbiome. In patients with primary immunodeficiency diseases that share the hallmark of atopic dermatitis, the skin displayed increased permissiveness with altered microbial population structures, decreased site specificity and colonization with species not found on controls. Increased fungal diversity and the increased presence of *Candida* and *Aspergillus* were consistent with the increased susceptibility of these patients to fungal infections (*Oh* et al., 2013). In mice, the systemic inhibition of complement signalling led to significant changes in the skin microbiota (*Chehoud* et al., 2013). This was concomitant with a decrease in pattern recognition receptors, antimicrobial peptides, cytokines and chemokines. These findings highlight the importance of host-microbe interactions in skin homeostasis. They also demonstrate that changes in host immunity can lead to changes in the composition of the microbiota. Considering the effect of vitamin D on adaptive and innate immune responses, exposure of the skin to sunlight could lead to changes in host immunity and in turn impact the composition of the microbiota.

UVB exposure either through sunlight or artificial light causes immune suppression in the skin (*Field* et al., 2005). Phototherapy containing UVB rays is used to treat both inflammatory conditions of atopic dermatitis and psoriasis. In the case of atopic dermatitis this can lead to a reduction of infection. In both conditions, a reduction in inflammation is observed with an increase in regulatory T cells and improvement of the epidermal barrier and restoration of cutaneous homeostasis (*Tartar* et al., 2014). As dysregulation of CAMP has been implicated in these disorders, it would be of great interest to determine if regulation of CAMP by vitamin D synthesized in the skin contributes to the restoration of homeostasis. It has been demonstrated that oral vitamin D supplements increased CAMP expression in the skin of atopic patients; however, improvement in barrier defence or changes in microbiota composition were not determined (*Hata* et al., 2008). Narrow-band UVB treatment of children with atopic dermatitis and controls with vitiligo caused a decrease in cutaneous *Staphylococcal* populations and in atopic patients the number of *S. aureus* strains that produced toxin were reduced to the levels found on the controls. This study

shows that UVB can cause shifts in the microbial composition of the skin, but did not address mechanism (*Manco* et al., 2006). The immune suppression caused by UVB is counterintuitive to the reduced infections in patients. The fact that UVB also induces CAMP expression may decrease inflammation while boosting the innate immune system and protecting the skin from infection (*Mallbris* et al., 2005; *Zasloff,* 2005).

THE VITAMIN D-CATHELICIDIN PATHWAY IS HUMAN AND PRIMATE-SPECIFIC

As discussed previously, the use of transgenic and knockout mouse models for immune mediators has provided insight between the interactions of the host and its microbiota, both in terms of host genes that impact the composition of the microbiota and how the microbiota impacts the host immune system. The vitamin D-cathelicidin pathway is human and primate-specific and not conserved in mice and other mammals (*Gombart* et al., 2005, 2009). Therefore, it would be difficult to model in animals. We generated a transgenic mouse line that carries a genomic copy of the human gene (manuscript in preparation). This mouse was then crossed on to the CAMP knockout mouse background (*Nizet* et al., 2001) to create a mouse that carries only the human CAMP gene. The human CAMP gene is expressed in epithelial barrier tissues and responsive to $1,25(OH)_2D_3$ *in vitro* and *in vivo* (manuscript in preparation).

We and others have identified an important difference between humans and mice in regards to vitamin D metabolism. It was recently reported that mouse macrophages do not express CYP27B1 even with LPS stimulation as observed in humans (*Kapetanovic* et al., 2012; *Ooi* et al., 2014). In mice, the source of CYP27B1 in the immune system was reported only in CD8+ T cells (*Ooi* et al., 2014). We have observed this lack of CYP27B1 expression in our "humanized" mouse model (manuscript in publication). Also, we found that it is not possible to induce vitamin D target genes like human CAMP with TLR activation in mouse macrophages in the presence of $25(OH)D_3$, although this occurs in humans as described previously (*Liu* et al., 2006). These findings would argue that utilization of vitamin D by the mouse immune system is very different from humans and that the mouse is not an ideal model with respect to vitamin D and the immune system. Nevertheless, the *CYP27B1* gene been reported to be expressed in the human and mouse skin (*Zehnder* et al., 2001; *Flanagan* et al., 2001; *Bikle* et al., 2004) with one report of no expression (*Kutuzova* et al., 2006). If the vitamin D pathway is better conserved between mice and humans in the skin, then we believe that our model could be useful for understanding the role of vitamin D and CAMP in skin barrier function and interactions with the microbiota. The mouse *Camp* knockout and "humanized" *CAMP* mouse should allow investigators to determine if the *CAMP* gene is important in determining the composition of the skin microbiota and may shed light on the role of sunlight and vitamin D on the health the skin and its commensals.

CONCLUSION

The role of sunlight and vitamin D on the form and function of the skin involves numerous mechanisms. It is clearly important in the differentiation of keratinocytes into the different layers that form the epidermis and the epidermal permeability layer. The homing of immune cells, the degree of inflammation and the expression of AMPs are all controlled by the vitamin D pathway. Wounding activates the vitamin D pathway and the up-regulation of pathogen sensors and the cathelicidin antimicrobial pathway are keys in fighting infection and promoting healing. The capacity of the skin to synthesize bioactive $1,25(OH)_2D_3$ and increase expression of the cathelicidin suggests that sun exposure may play a role in determining the composition of the microbiota; however, this remains to be determined. Animal models are not adequate as the vitamin D-cathelicidin pathway is conserved only in humans and non-human primates; therefore, either "humanized" mouse models or studies in humans are needed. We have developed a "humanized" cathelicidin transgenic mouse, but important differences between humans and mice with respect to the use of vitamin D by the immune system could limit its utility. Elucidating the importance of sunlight, vitamin D and its impact on the skin microbiome will require careful studies and recognition of limitations in both model systems.

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