

IL-17RA blockade is signaling through human IL-17RC, another receptor from the IL-17R family that can bind IL-17.

We noted strong cell-surface expression of IL-17R in lesional AD keratinocytes ($n = 5$) by immunohistochemistry, with a staining pattern that is consistent with normal keratinocytes ($n = 5$; Fig 2, A). In addition to keratinocytes, scattered dermal cells were noted to express IL-17R as previously described.² Consistent with immunohistochemical findings, gene expression levels for IL-17R measured by quantitative RT-PCR were similar between AD and normal keratinocytes (see this article's Fig E1 in the Online Repository at www.jacionline.org).

After establishing surface expression of IL-17R in AD keratinocytes, we assessed whether these cells can sufficiently respond to IL-17 stimulation by genomic analysis (see Methods in the Online Repository). Genes with fold change >1.5 and false discovery rate <0.1 in IL-17-treated versus untreated AD keratinocytes were considered differentially expressed (see this article's Table E1 in the Online Repository at www.jacionline.org). The AD gene set was subsequently compared against previously published IL-17-modulated genes in normal keratinocytes.²

We found strong positive correlation between IL-17-modulated gene sets generated from AD keratinocytes and normal keratinocytes ($r = 0.91$; Fig 2, B). The close similarity between AD and normal keratinocyte gene sets was further established by the computed connectivity score¹⁰ (0.904 ; $P < 10^{-16}$), wherein a perfect agreement between the 2 gene sets would yield a value of 1, whereas a perfect disagreement would be -1 . Importantly, AD keratinocytes stimulated with IL-17 had robust upregulation of AMPs *DEFB4* (517-fold from control) and *LCN2* (5.7-fold from control), similar to normal keratinocytes (Fig 2, B). Proinflammatory neutrophil chemoattractants, *CXCL1*, 3, 5, 6, and 8, as well as *CCR6*⁺ lymphocyte chemoattractant *CCL20* were likewise strongly induced in both AD and normal keratinocytes (Fig 2, B). This was verified by quantitative RT-PCR analysis showing that IL-17 significantly upregulated *DEFB4*, *LCN2*, *IL-8*, and *CCL20* expression in AD keratinocytes ($P < .005$) as it would in normal keratinocytes (Fig 2, C).

Despite increased IL-17 levels compared with normal skin^{8,9,11} and equivalent capacity to respond to cytokine stimulation, AD lesions still have minimal *DEFB4* and *LCN2* gene expression.⁹ To determine whether the T_H2 cytokine milieu within AD lesions may have an effect toward IL-17-induced AMP expression, we cultured normal keratinocytes with IL-17 (200 ng/mL) alone or in combination with IL-4 (200 ng/mL) or IL-13 (200 ng/mL) and examined for gene expression of the aforementioned AMPs. We found that both IL-4 and IL-13 can significantly antagonize IL-17 induction of *DEFB4* and *LCN2* in keratinocytes (Fig 2, D) as previously reported.¹² Thus, decreased innate defense molecule expression in AD is derived not from an inherent inability to respond to T_H17 cytokine stimulation but likely from low ligand levels, compounded by further suppressive action from the T_H2 microenvironment. Our findings have interesting implications for translational research because future therapeutics directed at increasing signaling through intact IL-17 receptors, despite low ligand expression, may potentially reverse this relative AMP deficiency in AD. One such approach might be T_H2 blockade, allowing existing IL-17 to function fully without suppression, potentially restoring AMP expression and alleviating microbial load in AD.

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UV-B-triggered induction of vitamin D3 metabolism differentially affects antimicrobial peptide expression in keratinocytes

To the Editor:

Antimicrobial peptides (AMPs) are important effectors in cutaneous innate immunity as antibiotics and signaling molecules in the defense against microbes.¹ The regulation of AMP expression and function in the skin is not completely understood. In the

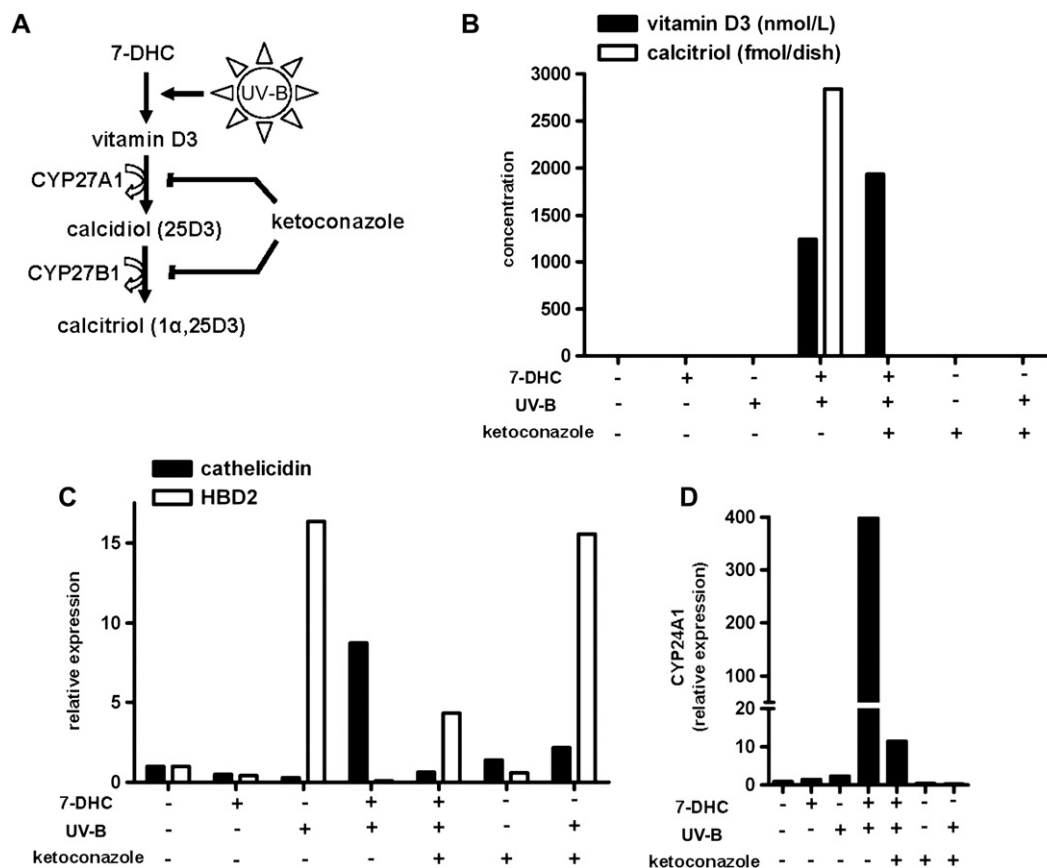


FIG 1. **A**, Schematic of calcitriol (1 α ,25-dihydroxyvitamin D3 [1 α ,25D3]) synthesis from 7-DHC. **B**, UV-B triggers the synthesis of vitamin D3 and calcitriol from 7-DHC in keratinocytes. **C and D**, UV-B induces HBD2 but has no effect on cathelicidin. In the presence of 7-DHC, expression of cathelicidin and CYP24A1 is increased after UV-B irradiation, whereas induction of HBD2 is blocked. Inhibition of calcitriol synthesis by ketoconazole rescues HBD2 induction and blocks cathelicidin and CYP24A1.

Journal, Gläser et al² recently reported in an elegant study that the AMPs human β -defensin (HBD)-2, HBD3, ribonuclease (RNase) 7, and psoriasin are induced in human keratinocytes on UV-B treatment *in vitro* and *in vivo*. In another recent study, expression of a different important AMP in skin—cathelicidin—was only enhanced after UV-B treatment *in vivo*,³ whereas in cultured primary human epidermal keratinocytes (NHEKs), no induction of cathelicidin after UV-B irradiation was observed.⁴ This observation could not be conclusively explained to date.

Cathelicidin expression in skin is induced by 1 α ,25-dihydroxyvitamin D3 (calcitriol) through activation of a vitamin D-responsive element in its gene promoter.¹ Notably, human keratinocytes possess the enzymatic machinery to produce calcitriol from the precursor 7-dehydrocholesterol (7-DHC)⁵ under the influence of UV-B irradiation. UV-B irradiation (290–315 nm) rapidly generates previtamin D3 from 7-DHC in these cells, and thermal isomerization subsequently generates vitamin D3, which is sequentially hydroxylated to yield calcidiol and finally calcitriol⁶ (Fig 1, A).

To investigate the *in vivo* and *in vitro* differences in cathelicidin regulation by UV-B treatment and the effect of increased vitamin D3 metabolism on AMP induction, experiments with NHEKs were performed. Keratinocytes (Cascade Biologics, Karlsruhe, Germany) were treated with vitamin D3 (10 nmol/L to 1 μ mol/L; Fluka, Munich, Germany), 7-DHC (25 μ mol/L; Sigma, Munich, Germany) and/or inhibitor ketoconazole (Paesel &

Lorei, Duisburg, Germany) in the presence of the solubilizer pluronic F127 (120 μ g/mL; Sigma) and irradiated with UV-B (300 nm; 30 mJ/cm²). Levels of vitamin D3 and calcitriol were analyzed by HPLC and ¹²⁵I-1 α ,25-dihydroxy vitamin D radio immuno assay (RIA) (IDS Ltd, Frankfurt, Germany) respectively. Cathelicidin, HBD2, CYP24A1, and CYP27B1 expression was analyzed by quantitative polymerase chain reaction (qPCR) as previously described.⁷ Cathelicidin peptide was investigated by Western blot (Innovagen, Lund, Sweden).

First we analyzed the generation of vitamin D3 and calcitriol from 7-DHC after UV-B treatment in cultured keratinocytes. NHEKs produced vitamin D3 from 7-DHC and generated calcitriol (Fig 1, B). Hydroxylation of vitamin D3 to form active calcitriol was blocked by the CYP inhibitor ketoconazole. To study whether activated vitamin D3 metabolism was sufficient to affect AMP expression, the calcitriol target genes cathelicidin and CYP24A1 were investigated: UV-B irradiation alone had no effect on both genes; however, when 7-DHC was present, cathelicidin and CYP24A1 transcript levels were induced. Ketoconazole inhibited the induction of both cathelicidin and CYP24A1 (Fig 1, C and D).

To ensure that UV-B treatment was effective, we determined expression levels of HBD2, which is known to be induced by UV-B in keratinocytes *in vitro* and slightly *in vivo*.² Upregulation of HBD2 by UV-B was confirmed; however, this effect was blocked when, at the same time, calcitriol synthesis from 7-DHC was

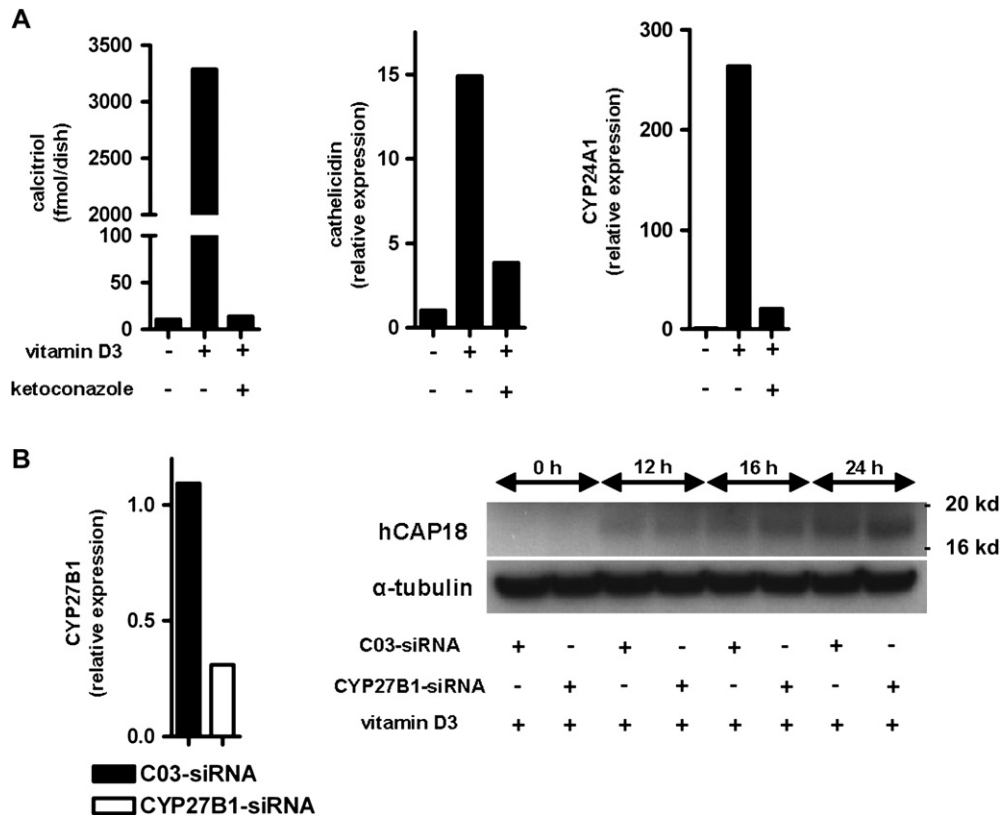


FIG 2. A, Keratinocytes produce calcitriol from vitamin D3 and subsequently induce mRNA expression of cathelicidin and CYP24A1. The CYP inhibitor ketoconazole inhibits these effects. B, Silencing of CYP27B1 by siRNA was analyzed by RT-PCR. Reduction of CYP27B1 does not prevent time-dependent induction of cathelicidin peptide by vitamin D3.

triggered by UV-B (Fig 1, C). Consequently, HBD2 induction was restored when vitamin D3 synthesis was inhibited by ketoconazole. Thus, higher calcitriol concentrations in the skin might lead to increased cathelicidin to strengthen the epidermal barrier and facilitate tissue repair.⁸ At the same time, increased vitamin D metabolism interferes with the induction of HBD2.

Because hydroxylation by CYP27A1 and CYP27B1 is required to activate vitamin D3 precursors, keratinocytes were incubated with vitamin D3, and production of calcitriol was measured. Indeed, increased levels of calcitriol on incubation with vitamin D3 were observed. Consequently, significantly increased cathelicidin and CYP24A1 expression in NHEKs was detected. Again, addition of CYP inhibitor ketoconazole led to reduced calcitriol production and gene expression levels (Fig 2, A).

We next tested whether changes in the expression of CYP27B1 were responsible for the higher levels of calcitriol in the supernatants and subsequent activation of transcription but found no differences (data not shown). To investigate further the role of CYP27B1, its expression was silenced by small interfering RNA (siRNA), but this did not result in reduced cathelicidin mRNA (data not shown) or human cathelicidin antimicrobial peptide 18 (hCAP18) peptide expression levels (Fig 2, B).

In a complementary approach, we treated NHEKs with precursor vitamin D3 alone. This resulted in highly elevated calcitriol levels and increased mRNA and protein levels of calcitriol target genes cathelicidin and CYP24A1. This confirms that keratinocytes are able to convert vitamin D3 by successive hydroxylation to hormonally active calcitriol. Importantly, this

system is so robust that silencing of CYP27B1 to about 30% of the initial activity does not inhibit production of calcitriol and subsequent induction of cathelicidin (Fig 2, B).

Thus, we demonstrate that human keratinocytes synthesize sufficient active calcitriol from 7-DHC to adjust cutaneous innate immunity after UV-B treatment *in vitro*. In particular, generation of calcitriol differentially affects AMP expression in keratinocytes: cathelicidin antimicrobial peptide is induced, and HBD2 induction is blocked. These findings complement the results by Mallbris et al,³ who showed that UV-B treatment of human skin directly up-regulated cathelicidin mRNA expression levels *in vivo* whereas *in vitro* UV-B had no effect on cathelicidin.⁴ We assume that these differences are a result of the presence or absence of vitamin D3 precursor 7-DHC. UV-B-induced calcitriol production involves conversion of 7-DHC to vitamin D3, a nonenzymatic photochemical process.⁹ Levels of 7-DHC in epidermal keratinocytes are high *in vivo*, whereas cultured keratinocytes have very low amounts. This is probably a result of an increased activity of the enzyme sterol Δ^7 -reductase *in vitro*, which converts 7-DHC to cholesterol that cannot be converted into vitamin D3 on UV-B treatment.¹⁰ Our results might also help explain the observation of "high and low responders" in the study published by Gläser et al.² Variable 7-DHC levels (and subsequently calcitriol levels) in the skin of the study patients could have differentially affected the induction of HBD2 after UV-B irradiation. Interestingly, in the experiments with skin explants—under standardized culture conditions without 7-DHC—Gläser et al² observed stronger induction of HBD2 compared with the *in vivo* part of their study.

In summary, we demonstrate that UV-B irradiation of human keratinocytes supplemented with 7-DHC triggers the synthesis of hormonally active calcitriol, which then differentially affects expression of AMPs cathelicidin and HBD2. Furthermore, we propose an explanation for the differences observed in AMP regulation *in vitro* and *in vivo* on UV-B treatment.

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Ovalbumin content in 2009 to 2010 seasonal and H1N1 monovalent influenza vaccines

To the Editor:

For the 2009 to 2010 influenza season, 34 manufacturers in 19 countries are manufacturing influenza vaccines. In the United

States, there are 5 manufacturers producing 1 nasal and 5 injectable Food and Drug Administration (FDA)–approved seasonal influenza vaccines. These manufacturers are also producing 1 nasal and 4 injectable H1N1 (hemagglutinin 1, neuraminidase 1) monovalent vaccines. Because influenza vaccines are developed by inoculating embryonated chicken eggs with virus, the final product has always contained ovalbumin, which can pose a risk in children and adults with egg allergy. However, because manufacturers do not report final lot ovalbumin content, allergists are directed to start with skin prick and intradermal skin testing to the vaccine followed by either a 2-dose or a graded multidose vaccine administration protocol.¹ However, these recommendations may be too conservative, because all but 1 manufacturer for the 2009 to 2010 FDA-approved influenza vaccines state a maximum ovalbumin content of ≤ 1 μ g per dose (≤ 2 μ g/mL), and improvements such as ultracentrifugation have resulted in lower ovalbumin content in vaccines like FluMist (MedImmune LLC, Gaithersburg, Md).²

The goal of this study was to determine the ovalbumin content in available 2009 to 2010 influenza vaccines. Because manufacturers express the maximum ovalbumin content as micrograms per dose, and there are 3 possible doses (0.2 mL for the nasal vaccines or 0.25 mL or 0.50 mL for the injectable vaccines), we chose a single concentration description of micrograms per milliliter to compare better the ovalbumin content of the vaccines against the manufacturers' stated content. A commercial ovalbumin ELISA kit (Chicken Egg Ovalbumin; Alpha Diagnostic International, San Antonio, Tex) that has a sensitivity range of 0.5 to 4 ng/mL was used. The detailed methods are provided in this article's [Methods](#) section of the Online Repository at www.jacionline.org. These results are presented in [Tables I and II](#).

In our opinion, this information is clinically important for 3 reasons: (1) manufacturers do not report final lot ovalbumin content, (2) routine measurement of ovalbumin is labor-intensive and generally inaccessible by practicing allergists, and (3) knowing the ovalbumin content guides current vaccine administration protocols. For children and adults with egg allergy, 3 clinical courses are generally recommended: vaccine avoidance, vaccine skin testing with a 2-dose vaccine administration protocol, or vaccine skin testing with a multidose graded vaccine administration protocol.¹

The 2-dose protocol (10% of the dose is given initially, and if there is no reaction in 30 minutes, the remaining 90% of the dose is given, with an additional observation period afterward) is based on a single study demonstrating the safety of this approach with vaccines containing no more than 1.2 μ g/mL ovalbumin in 83 children and adults with egg allergy.³ The multistep (usually 4-6 injections) graded challenge for patients with positive vaccine skin test results has been successful, although it has not been compared directly with a 2-step or single-step challenge.⁴ A recent abstract described success using a multidose protocol in 58 patients with egg allergy with positive vaccine skin test results, with only minor reactions, although the specific ovalbumin content was not reported.⁵ Also, since 1997, a virosomal adjuvanted influenza vaccine, Inflexal V (Berna Biotech, Berna, Switzerland), that is approved in Europe contains less than 50 ng of ovalbumin per dose. A recent study demonstrated the safety of this vaccine, administered as a single dose, in 44 children with egg allergy.⁶

Our study found the actual ovalbumin content of the brands and lots of vaccines tested from manufacturers claiming a maximum ovalbumin content of 2 μ g/mL was actually much lower than this level, specifically 0.001 to 0.007 μ g/mL for the