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Endogenous glucocorticoid deficiency in psoriasis promotes inflammation and abnormal differentiation

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Abstract

The factors involved in maintaining a localized inflammatory state in psoriatic skin remain poorly understood. Here, we demonstrate through metabolomic and transcriptomic profiling marked suppression of glucocorticoid biosynthesis in the epidermis of psoriatic skin leading to localized deficiency of cortisol. Utilizing a 3D human epidermis model, we demonstrate that glucocorticoid biosynthesis is suppressed by pro-inflammatory cytokines and that glucocorticoid deficiency promotes inflammatory responses in keratinocytes. Finally, we show *in vitro* and *in vivo* that treatment with topical glucocorticoids leads to rapid restoration of glucocorticoid biosynthesis gene expression coincident with normalization of epidermal differentiation and suppression of inflammatory responses. Taken together, our data suggest that localized glucocorticoid deficiency in psoriatic skin interferes with epidermal differentiation and promotes a sustained and localized inflammatory response. This may shed new light on the mechanism of action of topical steroids, and demonstrates the critical role of endogenous steroid in maintaining both inflammatory and differentiation homeostasis in the epidermis.

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The authors state no conflict of interest.

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INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder accompanied by increased proliferation and altered differentiation of epidermal keratinocytes. Topical glucocorticoids are a mainstay for mild-to-moderate psoriasis (Schon and Boehncke, 2005). It has been assumed that the effect of topical glucocorticoids is through anti-inflammatory action largely on infiltrating immune cells, but the precise cellular targets and mechanism of action of glucocorticoids in psoriasis remain unclear. The skin has been known to express the genes for enzymes involved in glucocorticoid biosynthesis (Dumont *et al*., 1992; Rogoff *et al*., 2001; Slominski *et al*., 1996; Slominski *et al*., 2004), and elements of the hypothalamo-pituitary-adrenal axis (HPA) have been implicated as major regulators of glucocorticoid synthesis in skin (Slominski *et al*., 2007). Loss of glucocorticoid receptor activity results in impaired epidermal differentiation, decreased expression of late differentiation markers including filaggrin, and abnormal hair growth (Bayo *et al*., 2008), features that are commonly seen in cutaneous psoriasis (Rittie *et al*., 2016). Furthermore, glucocorticoid receptor inactivation in mouse skin results in increased expression of various inflammatory markers (Sevilla *et al*., 2013), many of which are shared with psoriasis. Interestingly, reduced nuclear translocation of glucocorticoid receptors is a feature of psoriatic skin and keratinocytes (Man *et al*., 2013; Pang *et al*., 2015) but the reason for this has been unclear. Here, we show that endogenous biosynthesis of glucocorticoids is suppressed in psoriatic skin resulting in localized steroid deficiency. We further demonstrate that this steroid depleted microenvironment impacts epidermal differentiation and leads to heightened epidermal inflammatory responses, which can be normalized by exogenous glucocorticoids leading to restoration of epidermal differentiation, steroid biosynthesis, and inflammatory responses.

RESULTS

Profiling reveals marked metabolomic shifts with localized glucocorticoid deficiency in psoriatic skin

We performed untargeted metabolomic profiling using liquid chromatography followed by mass-spectrometry (LC-MS) on biopsies of lesional (PP) and uninvolved (PN) skin obtained from 6 psoriatic patients as well as normal (NN) skin from six healthy controls. Principal component analyses showed separation between lesional versus uninvolved and healthy control skin (Figure 1a). Among the 2724 metabolites examined, 377 were detected in at least two samples from each of the psoriatic and normal patients (Figure 1b). Amongst the most decreased compounds in PP skin were the glucocorticoids; cortisone and cortisol (357 and 1057-fold decreased, respectively, $p<1\times10^{-4}$) (Figure 2) (supplementary files 1 and 2). These data were validated for cortisol using a targeted steroid panel ($p<1\times10^{-3}$). Serum cortisol levels were slightly lower in psoriatic (n=37) compared to healthy controls (n=43), but this did not reach significance (p=0.08) (Figure 2c). Immunofluoresence (IF) showed decreased cortisol throughout the psoriatic epidermis compared to PN and NN skin (Figure 2d). These data demonstrate that psoriatic skin is in a locally confined state of chronic glucocorticoid deficiency. Other compounds dysregulated in psoriasis include taurine and homoserine (7,000- and 250- fold higher in psoriatic skin, respectively compared to uninvolved, $p<1\times10^{-4}$), and the B-vitamins riboflavin and pantothenate, which were 370-

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and 1600- fold lower, respectively, in psoriatic compared to uninvolved skin ($p<1\times10^{-4}$) (supplementary files 1 and 2).

Suppression of glucocorticoid biosynthesis in psoriasis and other skin disorders

Using RNA-seq, we characterized the transcriptomes of biopsies from the same plaques submitted for metabolomic profiling (n=6 in each group) (Supplementary Figure 1). Five out of six critical enzymes involved in steroid biosynthesis (Figure 3a, b) had decreased mRNA expression in psoriatic skin compared to both PN and NN skin. These included *HSD11B1*, which was reduced by 12-fold in PP compared to PN skin ($p=3.7\times10^{-5}$), and *HSD3B2*, which was reduced by 9-fold ($p=4\times10^{-3}$) (Supplementary Figures 2 and 3). We confirmed these findings at the protein level by immunohistochemistry (Figure 3c). Consistent with a glucocorticoid depleted microenvironment, we also observed decreased mRNA expression (1.4-fold, $p<0.05$) and decreased nuclear localization of the glucocorticoid receptor in PP skin (Figure 3d). (Figure 3d). Of note, as mineralcorticoid receptor has been implicated in glucocorticoid responses in skin (Boix *et al.*, 2016), mRNA expression of the mineralcorticoid receptor was also decreased in PP compared to PN and NN skin (2.2-fold decreased, $p<0.01$).

To assess for involvement of the HPA axis, we looked at the mRNA and protein expression of several genes involved in this pathway (Supplementary Figure 4). mRNA expression of cortitropin releasing hormone (CRH) and CRH-receptor-1 (CRHR1) was unchanged in both PP, PN and NN skin. Expression of the pro-opiomelancortin (POMC) was similarly unchanged between PP, PN and NN skin. These findings argue against the HPA-axis being responsible for the cortisol deficiency observed in psoriatic skin.

To address the character of genes regulated by glucocorticoid receptor in psoriatic skin, we retrieved the genomic locations of the binding sites for the glucocorticoid receptor gene (*NR3C1*) and focused on genes within 10 kilobases of at least one *NR3C1* binding site. Restricting the analysis to genes that are expressed in NN or PP skin, this generated a list of 3,453 genes (Heinz *et al.*, 2010). Given the ubiquitous binding of the glucocorticoid receptor, we limited our functional enrichment analysis to genes with at least 3 *NR3C1* binding sites. Highly enriched categories included cytokine signaling in immune system ($p<1\times10^{-4}$), response to type I interferons ($p=3\times10^{-4}$), response to IFN-γ ($p=1.8\times10^{-3}$), and differentiation (1.9×10^{-2}). This is consistent with the well-known anti-inflammatory effect of glucocorticoids in skin and further suggests a role for this steroid in epidermal differentiation (Supplementary file 3) (Stojadinovic *et al.*, 2007).

To address if suppression of glucocorticoid biosynthesis is unique to psoriasis we analyzed *HSD11B1* expression, as previously described by our group (Swindell *et al.*, 2016), across 41 datasets (17 psoriasis, 10 atopic dermatitis, 6 non-melanoma skin cancer, 6 wound healing, 2 acne, and discoid lupus). Decreased expression of *HSD11B1* was most profoundly suppressed in psoriasis ($p=5.4\times10^{-32}$), but closely followed by atopic dermatitis ($p=1.3\times10^{-8}$), wound healing ($p=1.1\times10^{-6}$) and squamous cell carcinoma ($p=2.26\times10^{-9}$) (Supplementary Figure 5 and file 4).

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Epidermal glucocorticoid biosynthesis and glucocorticoid consumption is dependent on differentiation stage

To gain a better understanding of the relative importance of local cortisol signaling axis in the epidermis, we utilized a 3D human skin tissue culture model comprised purely of normal human keratinocytes that differentiate with time into all of the stratified epidermal cell layers evident *in vivo* (Supplementary Figure 6). Importantly, this 3D tissue culture system is removed from any systemic hormone influence and is maintained in a growth medium that typically is supplemented with exogenous hydrocortisone (1000nM) (Arnette *et al*., 2016). Using this model, we found an increase in *HSD11B1* and *HSD11B2* mRNA expression with progression of epidermal differentiation ($p<0.05$) (Supplementary Figure 6) that strongly correlated with markers of differentiation (loricrin, $r=0.55$, $p<0.003$). Stimulation of the 3D rafts with pro-inflammatory cytokines, using TNF-α, IL-17A, and IL-22 individually, or in a combination for 72 hours led to decreased expression of both *HSD11B1* and *HSD11B2* mRNA with maximal suppression of *HSD11B1* mRNA expression from baseline observed with combination of TNF-α/IL-17A, with HSD11B1 being suppressed 1000-fold and *HSD11B2* about 15-fold ($p<0.0001$ and $p<0.01$ respectively) (Figure 3e). In contrast, no changes were seen with the same cytokines in monolayer keratinocyte cultures (data not shown), further supporting the role of epidermal differentiation in modulating inflammatory responses in context of glucocorticoid biosynthesis. Concomitant with the suppression of *HSD11B1* and *HSD11B2* expression, we observed decreased expression of epidermal differentiation markers, including involucrin ($p<0.05$), filaggrin ($p<0.01$) and loricrin ($p<0.01$), whereas inflammatory markers such as *IL36G* ($p<0.001$) and *DEFB4* ($p<0.001$) were increased (Figure 3e). Coincident with these changes, we found a differentiation-dependent decrease in total cortisol levels (2.9-fold decrease compared to baseline, n=3, $p<0.01$; Supplementary Figure 7). As HSD11B1 regulates synthesis of cortisol and HSD11B2 facilitates conversion of biologically active cortisol to inactive cortisone, we determined the ratio between *HSD11B1* and *HSD11B2* in the 3D epidermal culture models as compared to patient skin. Consistent with the idea that cortisol synthesis is favored under steady-state conditions, *HSD11B1:HSD11B2* ratio in normal skin was 4:1 while this ratio was markedly lower in psoriatic skin (0.4:1). In 3D epidermis, this ratio was lowest in undifferentiated tissue equivalents (0.1:1) and increased with progressive differentiation (0.5:1) albeit never to the levels of intact skin; this likely reflecting the actively regenerating state of these *ex vivo* culture models (Supplementary Figure 7). Collectively, these data indicate that cortisol consumption and expression of critical enzymes in glucocorticoid biosynthesis are tightly linked to the differentiation state of human epidermis.

Glucocorticoid deficiency alters epidermal differentiation and elicits heightened inflammatory responses

To address the impact of decreased glucocorticoids on epidermal differentiation, we grew 3D human epidermal cultures in the presence of progressively lower concentrations of hydrocortisone from the initial stages of their development at an air-liquid interface (1000nM, 100nM, 10nM and 0nM). A stratified epithelial tissue equivalent formed under all these conditions, but hydrocortisone depletion was associated with impaired epidermal morphogenesis. In particular, there was a marked disorganization of the epidermal cell layers as reflected by disrupted localization of junctional proteins involved in maintaining

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epidermal tissue integrity, including E-cadherin and desmoglein 1 (Dsg1), and late stage differentiation markers (filaggrin, loricrin). In contrast, glucocorticoid depletion was associated with aberrant development of the granular layer and abnormal stratum corneum (Figure 4a). Accordingly, there was a marked increase in filaggrin and loricrin mRNA transcript levels in 3D epidermal tissues grown in reduced hydrocortisone conditions. Glucocorticoid deficiency also led to increased mRNA expression and secretion of pro-inflammatory cytokines including *IL36G*, *CXCL8/IL-8*, *CXCL9* and *CXCL10* ($p<0.05$ for all) (Figure 4b–c), in addition to decreased cortisol levels (Figure 4d). Interestingly, *HSD11B1* expression was increased ($p<0.001$) while *HSD11B2* was significantly decreased ($p<0.01$), indicating that the 3-D epidermal cultures depleted of exogenous glucocorticoids respond by ramping up endogenous cortisol production machinery.

To confirm the importance of keratinocyte-derived cortisol biosynthesis for epidermal differentiation, we used a pharmacological inhibitor of HSD11B1 (PF915275) to block endogenous cortisol synthesis (Bhat *et al.*, 2008). For this purpose, 3D epidermal cultures were differentiated in the presence of hydrocortisone (1000 nM) for 6 or 9 days and then switched to medium lacking hydrocortisone to mimic a steroid-depleted tissue microenvironment; the cultures were treated with PF915275 at this time point. Epidermal morphology and differentiation was largely unaffected by this more acute depletion of exogenous hydrocortisone alone whereas combination of glucocorticoid depletion with pharmacological blockade of HSD11B1 markedly impaired epidermal architecture and the distribution of structural and differentiation proteins, including E-cadherin, Dsg1, loricrin, and filaggrin (Figure 5a). Importantly, these changes were normalized by re-addition of exogenous cortisol (100nM). QRT-PCR demonstrated decreased expression of involucrin (*IVL*) and filaggrin (*FLG*) ($p<0.05$), and increased *HSD11B1* but decreased *HSD11B2* mRNA expression ($p<0.05$) (Figure 5b). These data highlight the importance of the endogenous cortisol metabolism machinery for maintaining epidermal differentiation.

Steroid treatment of psoriasis in vivo leads to restoration of the glucocorticoid biosynthesis in skin

Three patients with stable chronic plaque psoriasis were treated with medium potency topical steroid (triamcinolone acetate 0.1%) for 7 days (n=3). There was a marked decrease in epidermal thickness compared to baseline (Figure 6a) along with decreased mRNA and protein expression of inflammatory markers (hBD2, CXCL9 and CXCL10) ($p<0.05$). These changes were accompanied by an increase in markers of normal epidermal differentiation (loricrin, filaggrin, $p<0.05$ both) (Figure 6b). Furthermore, the normalization of psoriatic histology was accompanied by increased expression of *HSD11B1* and *HSD11B2* ($p<0.01$) (Figure 6b), suggesting restoration of endogenous glucocorticoid biosynthesis and homeostasis in the epidermis. Similarly, treatment of healthy skin with topical steroids for only 24 hours led to suppression of inflammatory responses and induction of genes involved in keratinocyte differentiation (Supplementary Figure 8). These data demonstrate that topical treatment with steroids leads to simultaneous suppression of inflammatory responses, normalization of epidermal differentiation, and restoration of endogenous glucocorticoid biosynthesis.

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DISCUSSION

Psoriasis is marked by gross histologic alterations (Lowes *et al.*, 2014) that are accompanied by pervasive transcriptomic (Jabbari *et al.*, 2012; Li *et al.*, 2014; Swindell *et al.*, 2013) and proteomic (Lundberg *et al.*, 2015; Swindell *et al.*, 2015) changes in inflamed skin. However, global metabolic changes have not previously been addressed in psoriatic skin. Using liquid chromatography in tandem with mass spectrometry, we have identified striking metabolic shifts in psoriatic skin compared to both uninvolved and normal skin. These changes include profound deficiency of glucocorticoid hormones. Furthermore, by showing suppression of key enzymes in glucocorticoid biosynthesis, we provide evidence that this deficiency may not only be due to increased demand of glucocorticoids by the inflammatory infiltrate but also from decreased supply owing to localized suppression of endogenous glucocorticoid biosynthesis in the epidermis (Figure 2d). Notably, this explains previous observations showing impaired nuclear translocation of the glucocorticoid receptor in psoriatic skin (Man *et al.*, 2013) (Man *et al.*, 2013; Pang *et al.*, 2015), and provides important insights into the importance of glucocorticoid synthesis in the epidermis (Jozic *et al.*, 2014), and its role in inflammatory responses.

Glucocorticoids were first identified in the 1940s as potent anti-inflammatory agents (Lin and Wang, 2016). Since then, both natural and synthetic glucocorticoids have been among the most prescribed immune suppression treatments worldwide (Clark and Belvisi, 2012), and the mainstay of psoriasis treatment for over 50 years (Mrowietz and Domm, 2013). Glucocorticoids have been known to affect both proliferation and stimulate differentiation in the epidermis (Sugimoto and Endo, 1971; Sugimoto *et al.*, 1974), and human skin has been shown to express key enzymes involved in glucocorticoid biosynthesis, and being capable of producing glucocorticoids (Dumont *et al.*, 1992; Rogoff *et al.*, 2001; Slominski *et al.*, 1996; Slominski *et al.*, 2007; Slominski *et al.*, 2004). Our data extend these observations by demonstrating that expression of the glucocorticoid biosynthetic pathway is closely linked to epidermal differentiation (Figures 4–6), explaining why it has been difficult to detect *de novo* cortisol production by cultured monolayer keratinocytes (Milewich *et al.*, 1986; Slominski *et al.*, 2002).

Two enzymes, HSD11B1 and HSD11B2, are critical in regulating cortisol synthesis and both are expressed in skin. Whereas HSD11B1 activates cortisone to cortisol (Miller and Auchus, 2011; Slominski *et al.*, 2004; Tiganescu *et al.*, 2011), HSD11B2 converts cortisol to the inactive cortisone (Cirillo and Prime, 2011; Miller and Auchus, 2011; Tiganescu *et al.*, 2011; Vukelic *et al.*, 2011) (Figure 3). Thus, keratinocytes where HSD11B1 has been silenced produce less cortisol from cortisone than control cells (Cirillo and Prime, 2011). In contrast, silencing of *HSD11B2* leads to elevation of cortisol levels (Cirillo and Prime, 2011) demonstrating that the relative levels and activities of these two enzymes determine the overall levels of glucocorticoids locally present in the skin (Cirillo and Prime, 2011; Tiganescu *et al.*, 2011). In psoriatic skin, the mRNA expression of both *HSD11B1*, consistent with findings from a recent study (Terao *et al.*, 2016), and *HSD11B2* is decreased, but the ratio between these two enzymes is biased toward greater suppression of *HSD11B1* and therefore less production of cortisol. This is similar to what we observe in several other skin diseases and wound healing (Supplementary file 4).

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Loss of glucocorticoid receptor activity in mice has pleiotropic effects on skin and its appendages, including profound changes in epidermal differentiation (Bayo *et al.*, 2008). Our findings using a 3D human epidermal model support the notion that glucocorticoids directly impact keratinocyte differentiation. Remarkably, many of the features in the epidermis overlap with histopathological characteristics of cutaneous psoriasis (Rittie *et al.*, 2016). A notable difference between human psoriasis and the 3D epidermal cultures was observed when hydrocortisone supplementation was withdrawn from the culture model. Under these circumstances, the 3D epidermal tissues responded by increasing *HSD11B1* and lowering *HSD11B2* expression, likely in an attempt to drive up endogenous production of cortisol. As the steroid biosynthetic pathway is suppressed in psoriatic skin, this compensatory mechanism is probably not able to correct for cortisol deficiency. In addition, the glucocorticoid deficiency leads to heightened pro-inflammatory responses and increased expression of late differentiation markers in the epidermal tissue equivalents. When endogenous production of cortisol is blocked by pharmacological inhibitors, the expression of differentiation markers decreases following the pattern observed in psoriatic skin (Figure 6). These 3D skin culture data demonstrate that glucocorticoids have a critical role in epidermal biology by promoting differentiation and suppressing pro-inflammatory responses. Our *ex vivo* data support this notion by showing that application of topical glucocorticoids to lesional skin leads to rapid improvement with normalization of epidermal differentiation markers, decreased inflammation, and normalization of both *HSD11B1* and *HSD11B2* expression in order to restore endogenous glucocorticoid biosynthesis in the skin. Similarly, the application of steroid to healthy skin has similar effects many of which have been shown to have increased expression in clinically uninvolved skin of psoriatic patients (Gudjonsson *et al.*, 2009), consistent with the elevated cortisol levels in uninvolved skin (Figure 2).

The findings presented here provide additional insights into what is the cause of the suppression of glucocorticoid biosynthesis in psoriatic skin. Elements of the hypothalamopituitary- adrenal axis (HPA) have been implicated as major regulators of glucocorticoid synthesis in skin (Slominski *et al.*, 2007), however, we were unable to find evidence for their involvement in glucocorticoid suppression in psoriasis (Supplementary Figure 4). In keeping with the data presented here, a study looking at HPA-axis in patients with psoriatic arthritis and psoriasis failed to observe any correlation between ACTH levels and serum cortisol, or with PASI responses. Interestingly, and consistent with our data, serum cortisol levels increased as disease improved (Atzeni *et al.*, 2008). Our data demonstrate that the expression of *HSD11B1* and *HSD11B2* is closely related to the differentiation state of the epidermis (Figures 4–6), and that *HSD11B1* and *HSD11B2* expression in stratified epidermis is suppressed by the same pro-inflammatory cytokines that are known to be key drivers of psoriasis pathogenesis (Lowes *et al.*, 2014), including IL-17A and IL-22. These cytokines, particularly IL-22, disrupt epidermal differentiation (Sa *et al.*, 2007). Whether the down-regulation of *HSD11B1* and *HSD11B2* is related to the direct effect of these pro-inflammatory cytokines or secondary to the abnormal differentiation in psoriasis (Bernard *et al.*, 1985) remains to be investigated, but is an exciting avenue to address in future studies.

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In summary, these data expand our understanding of the role of glucocorticoids both in healthy and inflamed skin. Our study illustrates a role for endogenous production of glucocorticoids in epidermal differentiation and inflammatory responses, provide important insights into the pathobiology of psoriasis with implications for other chronic inflammatory conditions commonly treated with glucocorticoids, and may lead to novel therapeutic approaches targeting mechanisms governing glucocorticoid biosynthesis.

MATERIALS AND METHODS

Patient cohort

Nine patients with moderate chronic plaque psoriasis were enrolled for this study. Two biopsies were taken under local anesthesia from each psoriatic patient and each healthy control, with one biopsy submitted for RNA-sequencing and the other matching biopsy for metabolomic profiling. For the steroid treated cohort (n=3) patients underwent a single six millimeter punch biopsy prior to initiation of treatment and after 7 days of topical steroid use (triamcinolone acetate 0.1%). Informed written consent was obtained from all patients and controls, under protocols approved by the Institutional Review Board of the University of Michigan Medical School, and Northwestern University Institutional Review Board. This study was conducted according to the Declaration of Helsinki Principles.

Serum analyses and ELISAs

Serum was obtained from 37 patients with moderate-to-severe psoriasis and 43 healthy non-psoriatic controls. These samples were obtained at evaluation of patients recruited into our psoriasis genetic cohort database (Li *et al.*, 2014).

RNA-seq processing, QRT-PCR, statistical and bioinformatics analyses

RNA was isolated with RNeasy Plus Mini kit (Qiagen). 50 nucleotide single-end read was performed using Illumina Hi-Seq 2000 Genome Analyzer (Illumina). The reads were mapped by Cufflinks (Trapnell *et al.*, 2010) and TopHat (Trapnell *et al.*, 2009) and gene expression levels were expressed as fragments per kilobase of region per million mapped reads. For QRT-PCR RNA was reverse transcribed using a High Capacity cDNA Transcription kit (Applied Biosystems). qRT-PCR was performed using the 7900HT Fast Real-Time PCR system (Applied Biosystems) with Taqman primers purchased from Applied Biosystems (see supplemental methods)

Immunohistochemistry and immunofluorescence

Immunhistochemistry was performed on 5 μm thick paraffin sections from skin and epidermal rafts using antibodies (see supplemental methods)

Metabolomic profiling

Chromatography was performed on 1290 Infinity Binary LC System from Agilent together with Waters Acquity UPLC HSST3 1.8μm 2.1 × 100 mm column in connection with a Water Acquity UPLC HSS T3 1.8μm VanGuard Pre-column. Mass spectrometry was performed using Agilent Technologies 6530 Accurate-Mass Q-T of with a dual ASJ ESI ion source.

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Statistics

For all experiments described, 2-tailed Student's t tests were performed. Graphs are presented as mean ± SEM. P-values less than 0.05 were considered statistically significant. Raw data processing of metabolomic data was done using Agilent software (MassHunter Qual, and ProFinder).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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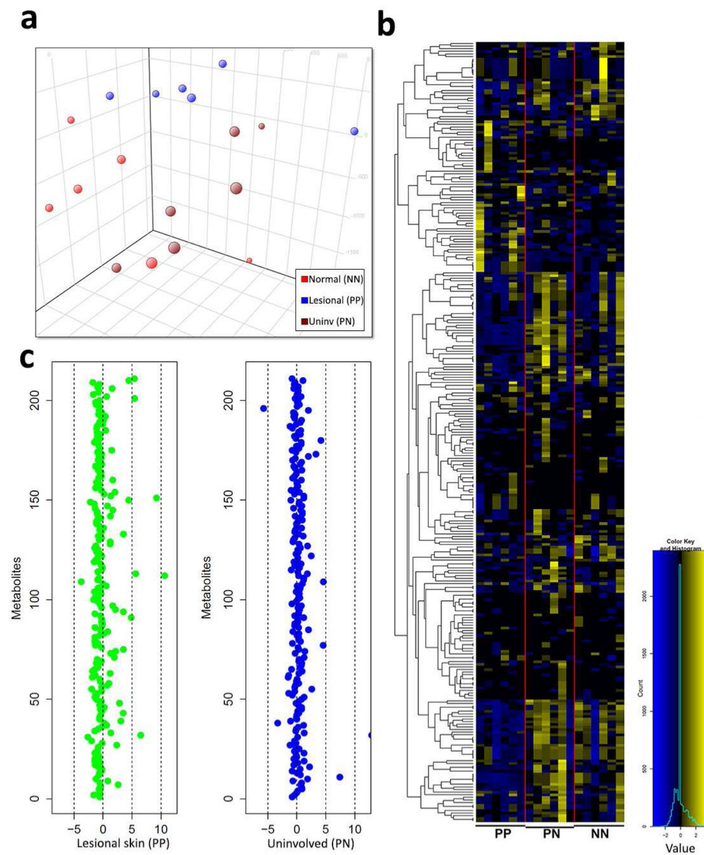

Figure 1. Metabolomic profiling reveals differences between psoriatic, uninvolved and normal skin

(**a**) Principal component analyses showed separation between lesional, uninvolved, and healthy control skin (n=6 for each). (**b**) Heatmap of named compounds identified in PP, PN, and NN skin. (**c**) Z-scores were calculated for both PP and PN skin for positively charged compounds.

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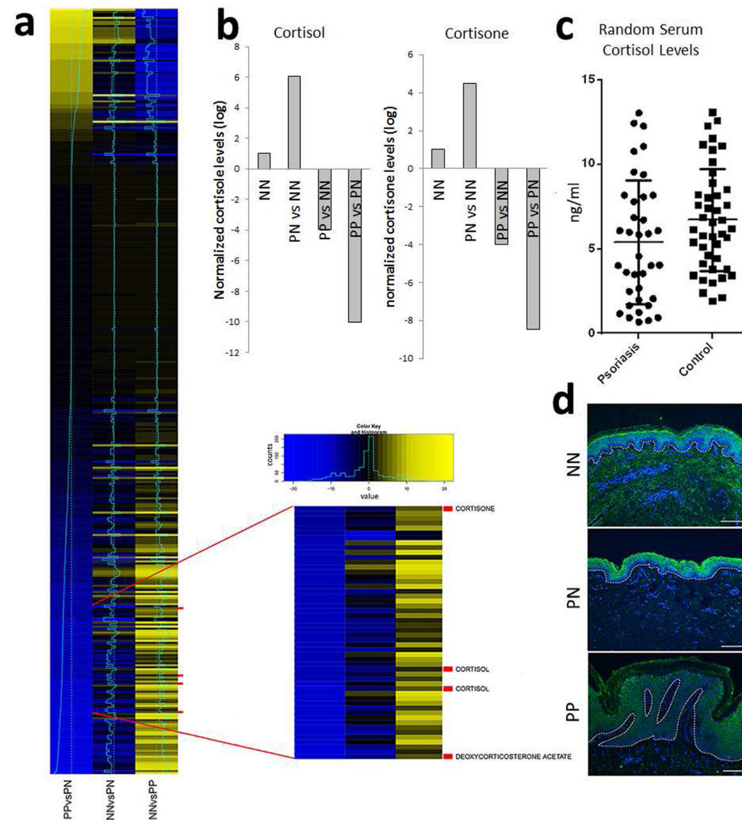

Figure 2. Glucocorticoid levels are decreased in psoriatic skin

(**a**) 377 metabolites were detected in at least two samples from each of the psoriatic and normal patients and significantly altered ($p<0.05$), and of the most decreased metabolites in psoriatic skin (both PP vs PN and PP vs NN) several were glucocorticoids including cortisone, cortisol and deoxycorticosterone acetate. (**b**) In comparison to normal healthy skin, glucocorticoid levels (both cortisol and cortisone) were markedly lower in psoriatic plaques compared to both NN and PN skin (log10 fold changes shown). (**c**) In contrast, random serum cortisol levels between psoriasis (n=37) and healthy controls (n=43) were slightly, but not significantly, lower in patients with psoriasis (p=0.08). (**d**) IF for cortisol showed decreased cortisol levels in psoriatic skin throughout the epidermis (n=3).

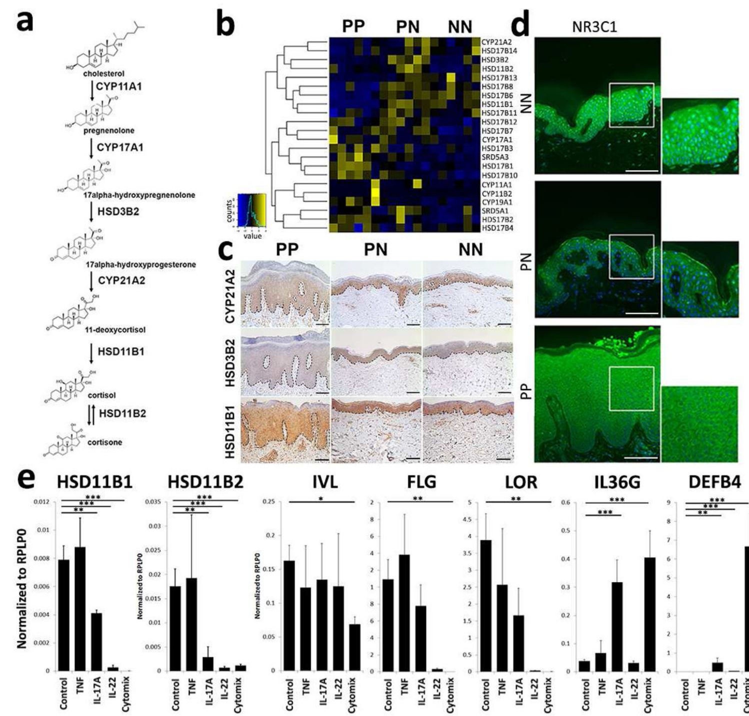

Figure 3. Glucocorticoid biosynthesis and steroid responses are suppressed in psoriatic skin

RNA-sequencing was performed on psoriatic (PP), uninvolved (PN) and normal (NN) skin (n=6) matching patients analyzed by metabolic profiling. (**a**) Glucocorticoid biosynthetic pathway. (**b–c**) Suppression of gene and protein expression involving key enzymes in the glucocorticoid biosynthetic pathway. (**d**) Reduced nuclear localization of the glucocorticoid receptor in psoriatic skin (scale bar = 100μm). (**e**) IL-17A and IL-22 at 10ng/ml suppressed *HSD11B1* and *HSD11B2* expression, whereas TNF-α (10ng/ml) had no effect in 3D epidermal raft cultures. Combination of IL-17A/IL-22 and TNF-α (cytomix) suppressed *HSD11B1* and *HSD11B2* expression, accompanied by decreased expression of desmoglein 1 (*DSG1*), filaggrin (*FLG*) and loricrin (*LOR*), but increased expression of *IL36G* and *DEFB4*. (Data shown with SEM, n=2–10, **p<0.01, ***p<0.001, for IHC and IF representative figures are shown (n=3)).

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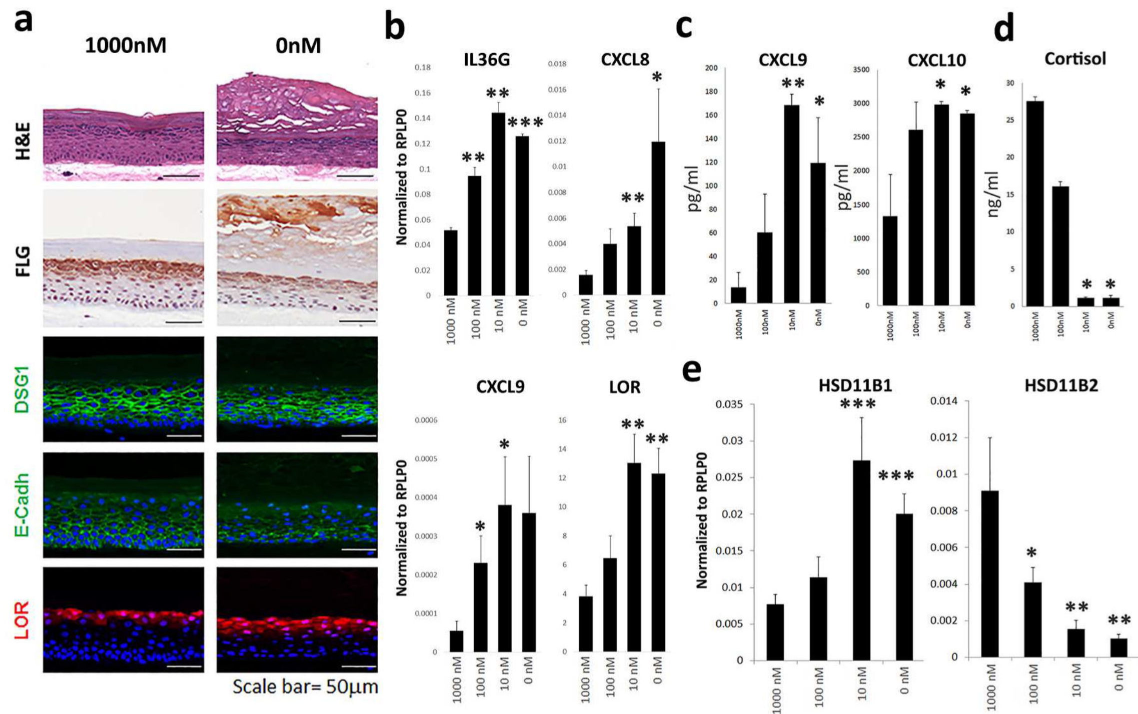

Figure 4. Glucocorticoid deficiency leads to abnormal epidermal differentiation and heightened pro-inflammatory responses

(**a**) 3D-human epidermal tissue cultures were grown in the presence of normal and low concentrations of hydrocortisone (1000nM, 100nM, 10nM and 0 nM). Histologically, this was associated with thickening of the epidermis with increased prominence of the granular layer and thickening of the stratum corneum, and expression of loricrin (LOR), and abnormal intercellular distribution of desmoglein-1 (DSG1) and E-cadherin (E-cad). (**b–c**) mRNA expression and protein secretion of pro-inflammatory and differentiation markers was increased with progressively lower hydrocortisone supplementation. (**d**) There was progressive decrease in cortisol levels, and decreased expression of *HSD11B2* (p<0.01), while **(e)** *HSD11B1* expression was increased (p<0.001). (Data shown with SEM, n=4, *p<0.05, **p<0.01, ***p<0.001, for IHC and IF representative figures are shown (n=3)).

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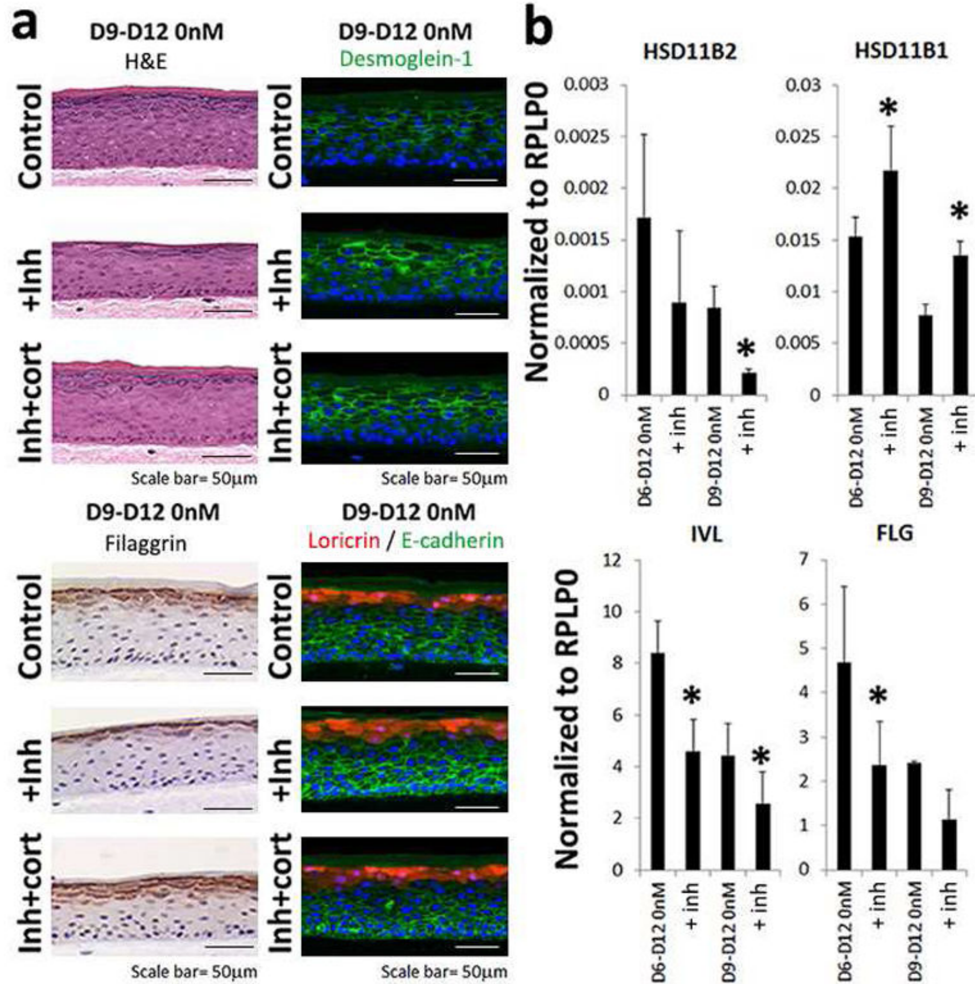

Figure 5. Exogenous glucocorticoids suppress inflammatory response and restore epidermal differentiation

(**a**) We grew 3D-human epidermis in the presence of hydrocortisone (1000nM) for 6 (data not shown) to 9 days and then switched the cultures to steroid deficient medium (0nM hydrocortisone) +/− the HSD11B1 inhibitor PF915275 (inh). Whereas control cultures showed near normal epidermal differentiation, cultures treated with the HSD11B1 inhibitor showed marked epidermal changes including filaggrin, desmoglein-1 and loricrin/E-cadherin protein expression. These changes were rescued by addition of exogenous cortisol (100nM). (**b**) QRT-PCR demonstrated decreased expression of involucrin (*IVL*), filaggrin (*FLG*) and *HSD11B2*, but increased *HSD11B1* mRNA expression. (Data shown with SEM, n=4, *$p<0.05$, for IHC and IF representative figures are shown (n=3)).

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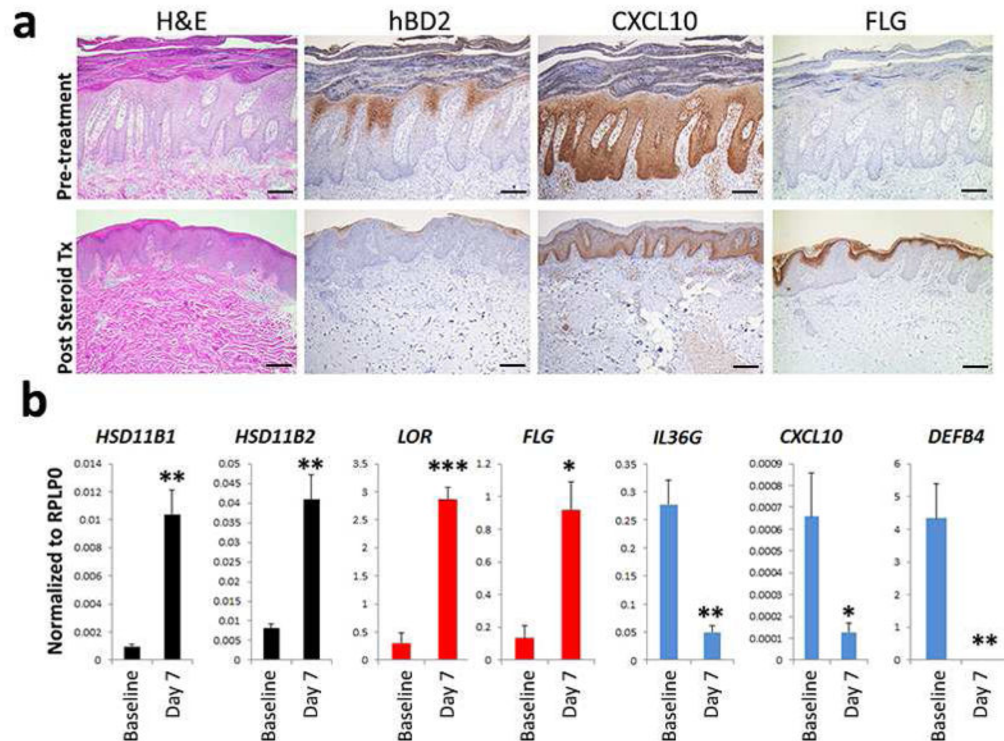

Figure 6. Topical glucocorticoids normalize epidermal differentiation and suppress inflammatory response *in vivo*

To address the role of topical glucocorticoids *in vivo,* patients with stable chronic plaque psoriasis were treated with medium potency topical steroid (triamcinolone acetate 0.1%) for 7 days (n=3). Compared to baseline there was marked decrease in epidermal thickness and decreased protein (**a**) and mRNA expression (**b**) of inflammatory markers, while markers of epidermal differentiation and HSD11B1/HSD11B2 mRNA expression normalized. (Data shown with SEM, n=4, *p<0.05, **p<0.01, ***p<0.001).