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Dysfunctional Skin-Derived Glucocorticoid Synthesis Is a Pathogenic Mechanism of Psoriasis

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Glucocorticoids (GC) are the primary steroids that regulate inflammation and have been exploited therapeutically in inflammatory skin diseases. Despite the broad-spectrum therapeutic use of GC, the biochemical rationale for locally treating inflammatory skin conditions is poorly understood, as systemic GC production remains largely functional in these patients. GC synthesis has been well characterized in healthy skin, but the pathological consequence has not been examined. Here we show de novo GC synthesis, and GC receptor expression is dysfunctional in both nonlesional and lesional psoriatic skin. Use of GC receptor epidermal knockout mice with adrenalectomy allowed for the distinction between local (keratinocyte) and systemic GC activity. Compensation exhibited by adult GC receptor epidermal knockout mice demonstrated that keratinocyte-derived GC synthesis protected skin from topical phorbol 12-myristate 13-acetate-induced inflammatory assault. Thus, localized de novo GC synthesis in skin is essential for controlling inflammation, and loss of the GC pathway in psoriatic skin represents an additional pathological process in this complex inflammatory skin disease.

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INTRODUCTION

Psoriasis affects 2% of the world's population, of which 80% suffer from mild forms of the disease that are commonly treated with topical therapies (Uva et al., 2012). Glucocorticoids (GC), often in combination with vitamin D or retinoids, are the primary topical therapeutics for mild-moderate psoriasis. GC, released as cortisol (humans) and corticosterone (rodents), are stress-response hormones with potent immunoregulatory mechanisms (Lightman et al., 2008). GC can suppress keratinocyte inflammation, downregulate proliferation, and promote differentiation (Stojadinovic et al., 2006). Because GC form an integral component of many topical psoriasis therapies, and healthy keratinocytes can synthesize GC de novo (Cirillo and Prime, 2011; Hannen et al., 2011; Vukelic et al., 2011; Wierzbicka et al., 2016), we hypothesize that loss of effective skin-GC synthesis would form a pathogenic mechanism of psoriasis.

Systemically, GC are produced via the hypothalamic-pituitary-adrenal (HPA) axis, forming an integral circadian rhythm and acutely in response to stress. In psoriasis, the systemic HPA axis remains largely unperturbed (Karanikas et al., 2009) and patients with psoriasis are not routinely prescribed systemic GC treatment. Healthy skin has a localized HPA axis, expressing all the components for regulating and synthesizing GC (Ito et al., 2005; Jozic et al., 2015; Slominski et al., 2007, 2014, 2015; Skobowiat et al., 2011; Skobowiat and Slominski, 2015; Wierzbicka et al., 2016; Zmijewski et al., 2007); however, the rate of GC synthesis is <1% of classic steroidogenic tissue (Slominski et al., 2004, 2015). Thus the significance relative to the abundant systemic supply from adrenal glands remains contested (Jozic et al., 2015).

Psoriasis is primarily considered an immune condition, but precisely why the immune system targets skin in psoriasis and is activated after environmental stress is unclear. The skin-HPA axis has been suggested as a stress-response mechanism (Reich et al., 2010; Slominski et al., 2013a, 2013b), and healthy

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Abbreviations: ADX, adrenalectomized; CON, control; CORT, corticosterone; GC, glucocorticoids; GR, glucocorticoid receptor; GREKO, GR epidermal knockout; HPA, hypothalamic-pituitary-adrenal; PMA, phorbol 12-myristate 13-acetate; Sham, sham operated

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human keratinocytes respond to stressors such as humidity (Takei et al., 2013), UV light (Skobowiat and Slominski, 2015), and trauma (Vukelic et al., 2011) by de novo synthesizing GC. Thus defective GC synthesis in psoriasis skin could form an additional mechanism to explain uncontrolled inflammation within lesional tissue and heightened sensitivity to disease flare from environmental cues. Here we assess the necessity of localized GC synthesis in skin independently of the adrenal gland, and its pathological consequences in psoriasis.

RESULTS

Steroid enzyme expression is reduced in psoriatic keratinocytes

RNA relative expression analysis of enzymes required for de novo cortisol synthesis was analyzed from Nair et al. (2009) using the NCBI GEO profiling database (http://www.ncbi.nlm.nih.gov/geo/) and revealed decreased StAR and 3βHSD1 ($P < 0.0001$) in psoriasis skin (Figure 1a). We previously reported an absence of StAR expression in psoriatic skin (Hannen et al., 2011), but we show here that 3βHSD1 protein was absent in nonlesional and lesional psoriatic epidermis. In addition, CYP11A1 and CYP17 proteins were significantly reduced in lesional psoriatic skin, although there was a small increase in CYP11A1 RNA expression in the psoriatic lesion (Figure 1b). Gene expression of 11βHSD1 and 11βHSD2, responsible for cortisol to cortisone interconversion, was also significantly reduced in lesional tissue (Figure 1a).

De novo cortisol synthesis is suppressed in psoriatic keratinocytes

De novo cortisol synthesis in primary healthy and psoriatic keratinocytes was assessed by [^{3}H]-pregnenolone radiometric assay (Figure 1c). More than 40% [^{3}H]-pregnenolone was metabolized to cortisol in healthy primary keratinocytes, confirmed by comigration with an authentic [1,2,6,7-^{3}H]-cortisol standard. Less than 10% of pregnenolone was metabolized to cortisol in uninvolved and involved psoriatic keratinocytes (Figure 1d). There was little metabolism of [^{3}H]-pregnenolone in involved keratinocytes. In uninvolved psoriatic keratinocytes, metabolic intermediates were still detected together with increased formation of highly polar steroid species correlating with sulfated steroids (Figure 1c and Supplementary Figure S1 online). Steroid sulfotransferase SULT2B1 was significantly elevated in uninvolved and involved psoriasis skin relative to healthy controls (Supplementary Figure S1). Sulfate conjugation is an excretion mechanism for steroid clearance (Mueller et al., 2015) and could account for the depletion of cortisol bioavailability in uninvolved psoriasis skin.

Cortisol secretion by whole-skin mounts, from normal and paired uninvolved and involved psoriatic skin, was assessed by liquid chromatography-tandem mass spectrometry. Cortisol secretion was decreased by >90% in uninvolved and involved lesional psoriasis skin compared with healthy skin (healthy skin 809.6 ± 120.4 ng/ml; uninvolved 63.1 ± 7.9 ng/ml; involved 67.7 ± 11.8 ng/ml psoriatic skin; $P < 0.01$, $n = 8$) (Figure 1e). Cortisone levels were also significantly ($P < 0.001$) lower in psoriatic tissue (normal skin 1,940.0 ± 436.3 ng/ml, uninvolved 272.5 ± 211.5 ng/ml; involved 320.6 ± 101.4 ng/ml psoriatic skin) (Figure 1f), indicating that decreased cortisol levels did not occur via shifts in 11βHSD1 shuttle activity. If changes in the direction of 11βHSD1 activity were responsible for depleted cortisol levels, a concomitant increase in cortisone levels would be expected, which was not observed here. The cortisol to cortisone ratio was still 1:2 respectively in both normal and psoriatic systems. Instead, these data suggest that decreased de novo synthesis with steroid sulfation is responsible for dramatic cortisol reduction in psoriatic keratinocytes.

Glucocorticoid receptor levels and activity are impaired in psoriatic keratinocytes

We assessed glucocorticoid receptor (GR) and active (GC/ligand-bound) phospho-Ser^{211} GRα (pGR) to determine whether decreased local cortisol production was associated with decreased GR phosphorylation. Relative expression analysis identified a significant reduction of GR in lesional psoriatic skin compared with healthy controls and nonlesional skin (Figure 2a). However, immunoblotting and immunofluorescence histochemistry showed that there was a significant ($P < 0.05$) downregulation of pGR and GR expression in uninvolved psoriatic skin (Figure 2). GR was undetectable in 18 of the 20 involved psoriatic skin biopsies by immunofluorescence histochemistry and was significantly ($P < 0.05$) reduced in immunoblotting analysis (Figure 2). Notably, there was no difference between uninvolved and healthy skin at the mRNA level; thus a reduction of GR protein expression in uninvolved tissue is either due to changes of translation or degradation. Because GR expression is essential for epidermal barrier integrity, reduced expression of GR in psoriatic skin could directly affect barrier function.

Skin-derived de novo glucocorticoid synthesis prevents topical phorbol 12-myristate 13-acetate-mediated inflammation

To examine the specific importance of local keratinocyte cortisol synthesis and associated GR response, we utilized keratinocyte GR knockout mice (GREKO; K5-cre//GR$^{loxP/loxP}$). Multiple keratinocyte steroid enzyme knockouts would be required to achieve the same effect as knocking out the GR to locate the GC-specific response; thus the GREKO mouse was more suitable for this initial study. To separate local from systemic GR responses, control (CON; 0cre//GR$^{loxP/loxP}$) and GREKO mice were adrenalectomized (ADX) or sham operated (Sham), to create four groups: (i) CON-Sham; (ii) CON-ADX; (iii) GREKO-Sham; (iv) GREKO-ADX. Mice were topically administered phorbol 12-myristate 13-acetate (PMA) (8 μg) to one side of the dorsal skin to examine differences in inflammation.

In the absence of PMA, there was little phenotypic difference and no difference in epidermal thickness in adult (week 15) CON and GREKO mouse skin, regardless of ADX (Figure 3a). In the presence of PMA, male adult CON-Sham and CON-ADX epidermis was severely disrupted with epidermal thickening and immune cell infiltration (Figure 3a); 50% of CON-Sham and 60% of CON-ADX mice exhibited regions of complete epidermal destruction. In comparison, GREKO-Sham showed no significant epidermal thickening relative to untreated controls and only one GREKO-ADX mouse exhibited increased skin thickness, depicted in the scatter plot distribution (Figure 3b). Epidermal destruction was not detected in GREKO-Sham and was only observed in 25% GREKO-ADX mice (Figure 3b). Inflammatory profiles of male CON PMA-treated dorsal skin showed induction of IL-1β, IL-6, tumor necrosis