Epidermal Inactivation of the Glucocorticoid Receptor Triggers Skin Barrier Defects and Cutaneous Inflammation

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The glucocorticoid (GC) receptor (GR) mediates the effects of physiological and pharmacological GC ligands and has a major role in cutaneous pathophysiology. To dissect the epithelial versus mesenchymal contribution of GR in developing and adult skin, we generated mice with keratinocyte-restricted GR inactivation (GR epidermal knockout or GR^{EKO} mice). Developing and early postnatal GR^{EKO} mice exhibited impaired epidermal barrier formation, abnormal keratinocyte differentiation, hyperproliferation, and stratum corneum (SC) fragility. At birth, GR^{EKO} epidermis showed altered levels of epidermal differentiation complex genes, proteases and protease inhibitors which participate in SC maintenance, and innate immunity genes. Many upregulated genes, including *S100a8/a9* and *Tslp*, also have increased expression in inflammatory skin diseases. Infiltration of macrophages and degranulating mast cells were observed in newborn GR^{EKO} skin, hallmarks of atopic dermatitis. In addition to increased extracellular signal–regulated kinase activation, GR^{EKO} newborn and adult epidermis had increased levels of phosphorylated signal transducer and activator of transcription 3, a feature of psoriasis. Although adult GR^{EKO} epidermis had a mild phenotype of increased proliferation, perturbation of skin homeostasis with detergent or phorbol ester triggered an exaggerated proliferative and hyperkeratotic response relative to wild type. Together, our results show that epidermal loss of GR provokes skin barrier defects and cutaneous inflammation.

Journal of Investigative Dermatology (2013) **133,** 361–370; doi:10.1038/jid.2012.281; published online 6 September 2012

INTRODUCTION

The glucocorticoid (GC) receptor (GR or *Nr3c1*) is a ligand-activated transcription factor that belongs to the nuclear hormone receptor superfamily and regulates gene expression through DNA-binding–dependent and –independent mechanisms (Revollo and Cidlowski, 2009; Nicolaides *et al.*, 2010). GR is ubiquitously expressed and mediates the biological and therapeutical effects of endogenous and synthetic GCs (Revollo and Cidlowski, 2009; Nicolaides *et al.*, 2010). The wide use of GC analogs in clinical practice relies on their great efficacy as anti-inflammatory agents, mostly due to the antagonism between ligand-activated GR and the pro-inflammatory NF-κB, AP-1, and signal transducer and activator of transcription (STAT) signaling pathways in many cell types (Clark, 2007; Liberman *et al.*, 2007; De Bosscher and Haegeman, 2009). GCs are currently used to treat inflammatory skin pathologies, such as atopic dermatitis (AD) and psoriasis, because of their anti-proliferative and anti-inflammatory actions in both immune cells and keratinocytes (Schäcke *et al.*, 2002; Elias, 2010). The mechanisms mediating GR therapeutical actions in skin disease have been widely studied, however, much less is known about the role of GR in skin physiology (Pérez, 2011).

In rodents, classical studies have shown that exogenous GCs promote epidermal barrier formation during development. Conversely, GC treatment of adult animals perturbs permeability barrier homeostasis, suggestive of unique roles for the GR in development and adulthood (Sheu *et al.*, 1991; Aszterbaum *et al.*, 1993; Hanley *et al.*, 1998; Kao *et al.*, 2003). We previously demonstrated that ubiquitous ablation of GR ($GR^{-/-}$) leads to major defects in mouse skin development with impaired keratinocyte differentiation, and augmented proliferation and apoptosis (Bayo *et al.*, 2008). Transcriptional profiling of $GR^{-/-}$ embryonic skin together with studies using cultured primary keratinocytes showed aberrant expression of genes encoding epidermal barrier proteins (Sevilla *et al.*, 2010). However, these analyses did not allow for discrimination of the keratinocyte-specific contribution of GR in skin development and pathophysiology. Moreover, $GR^{-/-}$ mice had alterations in the synthesis

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Abbreviations: AD, atopic dermatitis; E16.5, E18.5, embryonic age in days; EDC, epidermal differentiation complex; GC, glucocorticoid; GR, glucocorticoid receptor; GR^{EKO}, GR epidermal knockout; K5, K6, K10, keratin 5, 6, 10; P0, P2, postnatal age in days; PMA, phorbol ester; SC, stratum corneum; WT, wild type

Received 27 March 2012; revised 27 June 2012; accepted 27 June 2012; published online 6 September 2012

and secretion of steroids, which resulted in increased levels of ACTH and circulating corticosterone that may contribute to the skin phenotype (Tronche *et al.*, 1999; Bayo *et al.*, 2008). Above all, perinatal death of $GR^{-/-}$ mice precluded analyzing skin phenotype progression and whether developmental defects result in increased susceptibility to skin inflammation in the adult age, as described for other mouse models featuring epidermal barrier defects (Segre, 2006; Cork *et al.*, 2009; Elias, 2010; Roberson and Bowcock, 2010).

To resolve these issues, we generated a mouse model with constitutive GR inactivation restricted to keratinocytes (GR epidermal knockout or GR^{EKO} mice). In this report, we describe that GR^{EKO} mice exhibited defects in epidermal development and competence with stratum corneum (SC) fragility and degranulated mast cells in the dermis. At birth, GR^{EKO} mice showed upregulation of known markers of cutaneous inflammatory diseases associated with epidermal barrier defects. The changes in gene expression were concomitant with increased extracellular signal–regulated kinase (ERK) and STAT3 activation in GR^{EKO} newborn epidermis. Although the skin in adult mice had a milder phenotype than the newborn mice, it was hypersensitive to treatment with detergent (SDS) or phorbol ester (PMA). Our results show that GR is required in keratinocytes for normal skin development and homeostasis, and that in its absence mice develop phenotypic and molecular characteristics of inflammatory skin diseases treated by GCs.

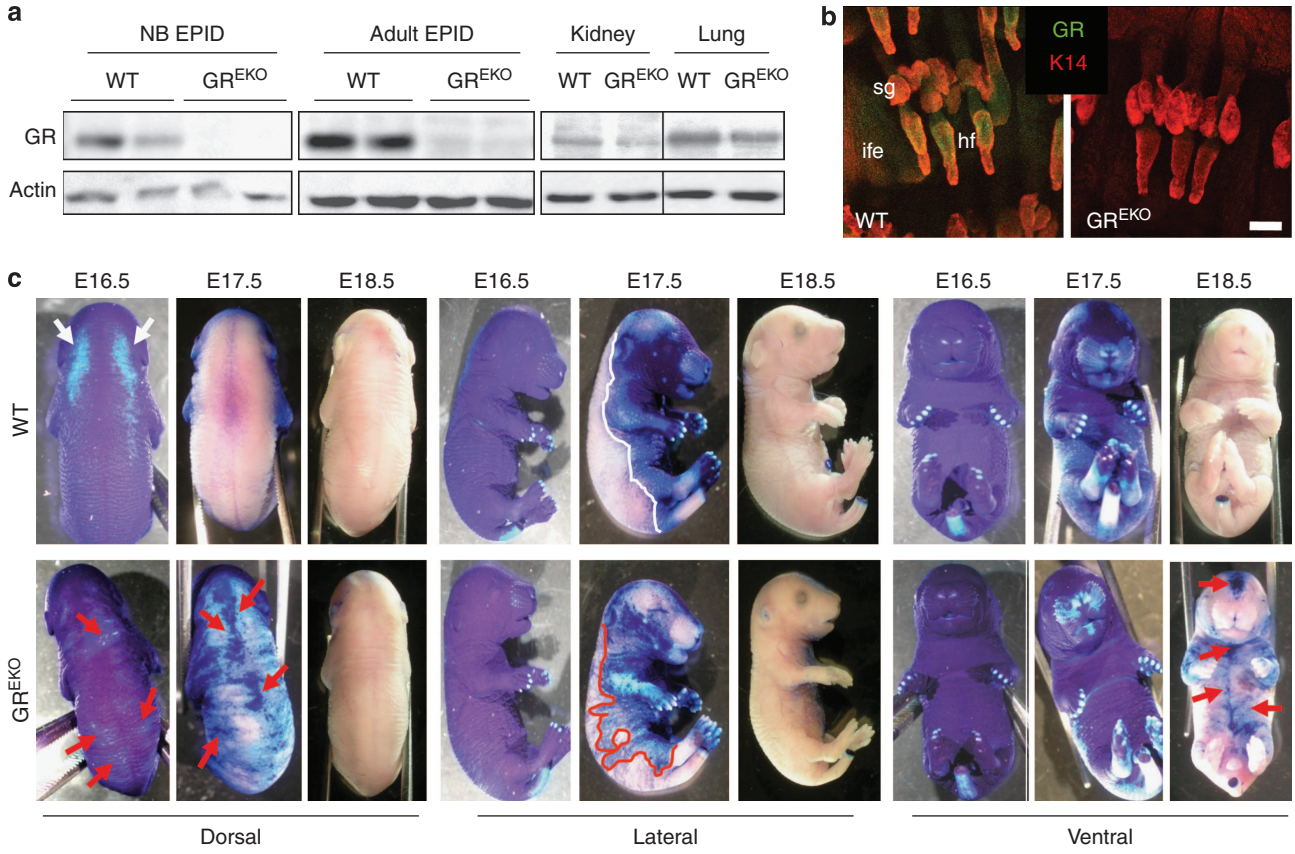

Figure 1. Mice with keratinocyte-restricted inactivation of glucocorticoid receptor (GR) (GR epidermal knockout (GR^{EKO})) exhibit impaired epidermal barrier formation. (**a**) Immunoblotting showing GR loss in epidermis (EPID) of newborn (NB) and adult mice but not in other tissues. (**b**) Immunofluorescence of tail epidermal whole mounts showing absence of GR in hair follicles (hf) and sebaceous glands (sg) in addition to interfollicular epidermis (ife). Bar = 100 μm. (**c**) Impaired epidermal barrier formation of GR^{EKO} mice. Epidermal maturation assessed by toluidine blue staining in late (E16.5 to E18.5) GR^{EKO} and wild-type (WT) embryos. GR^{EKO} mice showed delayed and altered epidermal barrier formation (red arrows) that did not follow the dorsoventral and anteroposterior pattern of WT mice (white arrows). Lines indicate the progression of epidermal maturation in WT (white) versus mutant (red) mice.

Figure 2. Abnormal epidermal differentiation and proliferation in glucocorticoid receptor epidermal knockout (GR^{EKO}) newborn mice. (**a**) Hematoxylin and eosin (H&E)–stained GR^{EKO} and wild-type (WT) postnatal (P) skin sections. Dotted line: epidermal (epi)–dermal (der) border. Arrows: hyperkeratosis. Asterisk: stratum corneum (SC) detachment. Bar = 100 μm. (**a′**) Epidermal thickness quantitation; Student's *t*-test; $*P<0.05$; $**P<0.005$. (**b**–**f**) P0 skin samples. (**b**) Electron microscopy; asterisks: SC detachment. Bar = 20 μm (left), 3 μm (right). (**c**) Nile red staining; polar (red) and nonpolar (green). Bar = 100 μm. (**d**) Immunostaining. CDSN, corneodesmosin; FIL, filaggrin; K6, keratin 6; LOR, loricrin. Black thick arrows: specific staining. White arrows: lack of staining. Thin arrows: parakeratosis. Asterisks: SC detachment. Bar = 100 μm (H&E), 50 μm (immunostaining). (**e**) BrdU-positive keratinocytes ($n = 12$). Mean values ± SD are shown. Student's *t*-test; $*P<0.05$. (**f**) Immunoblotting.