OVOL1 Regulates Psoriasis-Like Skin Inflammation and Epidermal Hyperplasia

Peng Sun\(^1\), Remy Vu\(^1,2\), Morgan Dragan\(^1,2\), Daniel Haensel\(^1,2\), Guadalupe Gutierrez\(^1\), Quy Nguyen\(^1\), Elyse Greenberg\(^1\), Zeyu Chen\(^1,3,4\), Jie Wu\(^1\), Scott Atwood\(^5\), Eric Pearlman\(^6\), Yuling Shi\(^4,7\), Wei Han\(^8\), Kai Kessenbrock\(^1\) and Xing Dai\(^1,2\)

Psoriasis is a common inflammatory skin disease characterized by aberrant inflammation and epidermal hyperplasia. Molecular mechanisms that regulate psoriasis-like skin inflammation remain to be fully understood. Here, we show that the expression of Ovol1 (encoding ovo-like 1 transcription factor) is upregulated in psoriatic skin, and its deletion results in aggravated psoriasis-like skin symptoms following stimulation with imiquimod. Using bulk and single-cell RNA sequencing, we identify molecular changes in the epidermal, fibroblast, and immune cells of Ovol1-deficient skin that reflect an altered course of epidermal differentiation and enhanced inflammatory responses. Furthermore, we provide evidence for excessive full-length IL-1\(\beta\) signaling in the microenvironment of imiquimod-treated Ovol1-deficient skin that functionally contributes to immune cell infiltration and epidermal hyperplasia. Collectively, our study uncovers a protective role for OVOL1 in curtailing psoriasis-like inflammation and the associated skin pathology.


INTRODUCTION

Psoriasis is one of the most common inflammatory skin diseases, affecting 2–3% of the world population (Ayala-Fontanez et al., 2016). The etiology of the disease is multifactorial, encompassing both genetic and environmental components that cause dysregulations of the skin barrier and immune cell function (Benhadou et al., 2019; Bergboer et al., 2012; Lowes et al., 2014; Roberson and Bowcock, 2010). Central to skin barrier function is the epidermis, a self-renewing epithelium composed of a basal layer containing stem and progenitor cells and multiple suprabasal layers containing progressively differentiating cells that produce stratum corneum, the actual physical barrier at the skin’s outermost surface (Gonzales and Fuchs, 2017). In psoriasis and other aberrant skin inflammatory processes, epidermal cells both serve as the target of immune cells and play a crucial role in shaping the immune responses (Kobayashi et al., 2019; Pasparakis et al., 2014). The molecular mechanisms that regulate the complex cross-talk between epidermal cells and immune cells in psoriasis remain to be fully elucidated. SNPs in OVOL1 have been shown to associate with several inflammatory skin diseases, namely atopic dermatitis, atopic march, and acne (Hirota et al., 2012; Kang et al., 2015; Marenholz et al., 2015; Navarini et al., 2014; Paternoster et al., 2011). OVOL1 encodes a zinc finger transcription factor, and its mouse homolog Ovol1 is functionally required for proper embryonic epidermal development (Dai et al., 1998; Lee et al., 2014; Nair et al., 2006; Teng et al., 2007). Specifically, Ovol1 is expressed in the transiently proliferative epidermal intermediate and spinous cells, and its germline ablation results in a thickened epidermis with expanded early differentiating cells (spinous layers) and a transient delay in barrier acquisition that is resolved by birth. Studies using cultured human keratinocytes (KCs) and clinical samples have implicated a possible functional involvement of OVOL1 in atopic dermatitis (Furue et al., 2019; Tsuji et al., 2020, 2017). Specifically, OVOL1 was shown to regulate the expression of epidermal terminal differentiation genes FLG and LOR. However, the in vivo function of Ovol1/OVOL1 in skin homeostasis and inflammation has not been addressed.

Despite the long-held view of distinct mechanisms underlying each inflammatory skin disorder, overlapping molecular mechanisms that affect both psoriasis and atopic dermatitis exist (Guttman-Yassky and Krueger, 2017). In this work, we report elevated Ovol1/Ovol1 expression in lesional psoriatic human skin and in epidermal cells of mouse skin with psoriasis-like inflammation. We show that Ovol1 is largely dispensable for adult epidermal homeostasis, but its deletion significantly aggravates imiquimod (IMQ)-induced psoriasis-like skin defects featuring epidermal hyperplasia and neutrophil accumulation. Using bulk and single-cell (sc) RNA sequencing (scRNA-sequencing) coupled with functional studies, we identify aberrant IL-1\(\beta\) expression and signaling as an
important mediator of the exacerbated inflammation and epidermal hyperplasia in Ovol1-deficient mice.

RESULTS

Ovol1 expression is upregulated in psoriatic skin

To seek clues for a functional involvement of Ovol1 in psoriasis, we examined our previously published RNA-seq data on human psoriasis skin samples (Yu et al., 2020). Ovol1 expression was found to be upregulated by >2-fold in psoriatic skin compared with normal skin (Figure 1a). We also interrogated a public dataset on paired nonlesional and lesional skin from human patients with psoriasis (Correa da Rosa et al., 2017; Suárez-Fariñas et al., 2012) and observed significantly higher Ovol1 expression in the lesional skin (Figure 1b). To determine if this is the case in an animal model of psoriasis, we turned to IMQ-treated mouse back skin. IMQ is a toll-like receptor 7/8 agonist and its short-term (5- to 7-day-long) application induces dermatitis with phenotypic and histological resemblance to human psoriasis lesions, including hyperkeratosis, erythema, scaling, neutrophil microabscesses in epidermis, and infiltration of γδ T cells and T helper type 17 cells (Gilliet et al., 2004; Schön et al., 2006; Sumida et al., 2014; Swindell et al., 2017; van der Fits et al., 2009; Wu et al., 2004). We generated Ovol1-LacZ mice that express β-galactosidase downstream of the Ovol1 promoter and found that IMQ treatment results in visibly increased β-galactosidase activity in both interfollicular epidermis and hair follicles (Figure 1c). Thus, Ovol1 expression is upregulated in psoriatic skin of both human and mouse.

We previously showed that calcium, which is known to induce epidermal differentiation, induces Ovol1 expression in mouse keratinocytes (Dai et al., 1998). Using human keratinocytes isolated from neonatal foreskin, we found that calcium increased the level of Ovol1 transcript by ~3-fold, but calcium together with IMQ elicited a ~10-fold increase (Figure 1d). This finding suggests that IMQ can directly upregulate Ovol1 expression in epidermal cells.

Ovol1 deficiency aggravates IMQ-induced epidermal hyperplasia and inflammatory response

We next investigated Ovol1 function in adult skin by analyzing Ovol1−/− mice on a CD1 outbred background because of perinatal lethality in a C57BL/6 strain background (Dai et al., 1998; Nair et al., 2006). Histology of, and number of Ki67+ proliferative cells in, Ovol1−/− epidermis were largely similar to those in the littermate control epidermis (Figure 2a–c). However, a slight increase in the ratio between suprabasal and basal cells in Ovol1−/− mice compared with the controls (Figure 2d–f), suggesting a very mild alteration in KC differentiation.

Next, we asked whether Ovol1 deletion impacts IMQ-induced skin inflammation and the associated epidermal hyperplasia. Over a 6-day course of IMQ treatment, psoriasis-like features including erythema and scaling were significantly more pronounced in Ovol1−/− mice than in control littermates (Figure 3a and b; Supplementary Figure S1a). H&E

Figure 1. Ovol1 expression is upregulated in psoriatic skin. (a) Ovol1 expression in psoriatic PS taken from each patient and control NN skin samples obtained from surgical discard specimens of healthy people. n = 5. (b) Ovol1 expression in PL and PN skin of human patients with psoriasis. n = 81. (c) X-gal staining of β-galactosidase activity in skin of Ovol1-LacZ mice, n = 2–3 (shown are images from 8-week-old mice). Bar = 100 μm. (d) RT-qPCR analysis of the indicated genes in human foreskin keratinocytes following calcium (1.2 mM for 30 hours) and IMQ (50 μg/ml for 6 hours) treatment. Data are from three independent experiments. ***p < 0.005, *p < 0.05. D, day after IMQ treatment; IMQ, imiquimod; IVL, involucrin; NN, normal nonpsoriatic; PL, paired lesional; PN, paired nonlesional; PS, plaque skin.
and immunostaining analysis revealed that IMQ-treated Ovol1−/− epidermis launched a hyperproliferative response faster than control littermates (Figure 3c–e). IMQ-induced defects in terminal differentiation were also more prominent in Ovol1−/− skin, shown by lower levels of the differentiation marker K10 (Figure 3f). Moreover, the aberrant upregulation of LM332 protein production as an indicator of tissue repair (Iorio et al., 2015) was detected earlier in the skin of IMQ-treated Ovol1−/− mice compared with control littermate skin (Figure 3g; Supplementary Figure S1b). Flow cytometry performed on whole skin revealed a 3.5-fold increase in CD45+ (immune) cells, a 16.5-fold increase in CD45+/Ly6G+/CD11b+ neutrophils, and a trending increase in CD45+/F4/80+/CD11b+/Ly6G− macrophages in IMQ-treated Ovol1−/− skin compared with control littermate skin (Figure 3h; Supplementary Figure S1c). Together, these data indicate that Ovol1 loss leads to rapid and severe psoriasis-like symptoms in response to IMQ.

Significantly elevated inflammatory gene expression in IMQ-treated Ovol1−/− epidermis

To elucidate the molecular nature of Ovol1 deficiency–induced changes in skin, we performed RNA-seq analysis on dispase-isolated epidermis from Ovol1−/− mice and control littermates that were either untreated or 6 hours after a single IMQ treatment (when epidermal hyperproliferation was not yet apparent; Figure 3d). Principle component analyses revealed that IMQ treatment and sex of the mice analyzed are major sources of variation in gene expression (Figure 4a; Supplementary Figure S2a). Dramatic differences in gene expression between IMQ-treated Ovol1−/− and control littermate epidermis were observed (Figure 4a and b; Supplementary Table S1). Molecular differences between untreated Ovol1−/− and control epidermis were also detected but were considerably less prominent than those with IMQ treatment (Figure 4a and b; Supplementary Table S1).

To identify the associated biological processes and pathways, we annotated genes differentially expressed between Ovol1−/− and control epidermis using Molecular Signatures Database to reveal the hallmark gene sets and using Enrichr to probe the GO_Biological_Process_2018 library (Chen et al., 2013; Kuleshov et al., 2016) (Supplementary Table S2). Gene signatures related to “inflammatory response” and “regulation of inflammatory response” were upregulated in IMQ-treated and untreated Ovol1−/− epidermis, respectively, relative to control counterparts (Figure 4c–e). Also enriched in IMQ-treated Ovol1−/− epidermis were Gene Ontology (GO) terms of “TNF-α signaling”, “cytokine-mediated signaling pathway”, “chemokine-mediated signaling pathway”, “regulation of cell proliferation”, “skin development”, and “keratinocyte differentiation” (Figure 4d). Specific upregulated genes in these GO terms (e.g., Tgfa, Slpi, Aqp3, Ccl20, Krt16, and Lce3c/d/e) are also upregulated in human psoriatic skin (Supplementary Table S3; Figure 1a). A number of inflammatory cytokine and chemokine genes, such as Il1a (3.6×, P < 10−16) and Tgfa (2.3×, P = 0.0001), were upregulated in IMQ-treated Ovol1−/− epidermis, whereas the expression of known psoriasis-associated cytokine genes Il17, Il23, Il1b, and Il6 was not affected at this early time point. Moreover, the expression of Flg and Lor, whose human counterparts are known to be regulated by OVOL1 (Furue et al., 2019; Tsuji et al., 2017), was not significantly affected by Ovol1 deletion in untreated and IMQ-treated epidermis. GO terms that
were more enriched in IMQ-treated control littermate epidermis compared with Ovol1−/− epidermis included “interferon alpha response”, “cholesterol homeostasis”, “interferon gamma response”, “skin development”, and “keratinocyte differentiation” (Supplementary Figure S2b and c). GO terms that were more enriched in untreated control epidermis than Ovol1−/− epidermis included “positive regulation of cAMP metabolic process” and “interleukin-18-mediated signaling pathway” (Supplementary Figure S2d). Taken together, our bulk RNA data identify enhanced inflammation and altered KC differentiation as major effects of Ovol1 loss in epidermis.
scRNA-seq analysis reveals altered gene expression in epidermal basal, suprabasal, fibroblast, and immune cells of the IMQ-treated Ovol1−/− skin.

Next, we molecularly profiled the skin of Ovol1−/− and Ctrl littermates at 24 hours after IMQ treatment, but this time using the 10X Genomics scRNA-seq platform to achieve a single-cell resolution. After quality control (Supplementary Materials and Methods; Supplementary Figure S3a), a total of 23,395 cells from four distinct conditions (6,289, 5,553, 5,857, and 5,696 cells from untreated control littermate, Figure 4. Gene expression in Ovol1−/− and Ctrl littermate epidermis. (a) PCA showing epidermal gene expression differences between untreated and IMQ-treated Ovol1−/− and Ctrl littermates. (b) Volcano plots showing DE genes between Ctrl and Ovol1−/− epidermis from untreated and IMQ-treated mice (n = 2 per genotype: 1 male and 1 female). Genes were filtered for subsequent analysis using the following cut-off: fold change > 2; adjusted P-value < 0.05. (c, d) Functional annotation of genes that were upregulated in IMQ-treated Ovol1−/− epidermis using Hallmark gene sets provided by (c) MSigDB and (d) GO terms in Biological Process 2018. (e) GO terms associated with genes upregulated in untreated Ovol1−/− epidermis. Shown are the top 10 functional terms and the vertical lines correspond to p = 0.05. Ctrl, control; DE, differentially expressed; GO, Gene Ontology; IMQ, imiquimod; MSigDB, Molecular Signatures Database; PCA, principal component analysis.
untreated $Ovol^{+/+}$, IMQ-treated control littermate, and IMQ-treated $Ovol^{+/+}$ mice, respectively) were aggregated for subsequent analysis (Supplementary Figure S3b). In the overall t-distributed stochastic neighbor embedding plot, the major cell types identified were epithelial cells ($Krt14^+$ or $Krt1^+$; 42.4%) and fibroblasts ($Col1a2^{high}$; 46.6%) (Figure 5a, top, and 5b; Supplementary Figure S3b and c). Epithelial cells or fibroblasts from untreated $Ovol^{+/+}$ skin intermingled with the same cell type from untreated control littermate skin, whereas IMQ elicited differential gene expression resulting in the formation of clusters of epithelial cells or fibroblasts that were distinct from the IMQ-treated $Ovol^{+/+}$ and control littermate skin (Figure 5a, bottom).

Given that $Ovol1$ is predominantly expressed in epidermal suprabasal cells, $Krt14^{high}$ (suprabasal spinous cell) clusters from each sample (untreated or IMQ-treated control and $Ovol1^{+/+}$) were computationally isolated, and those from all four conditions were aggregated together in a combined dataset (Supplementary Figure S3b). Suprabasal cells from the two untreated genotypes were well mixed in cluster 0, but...
those from IMQ-treated control skin were enriched in cluster 1 and those from IMQ-treated Ovol1−/− skin were enriched in cluster 2 (Figure 5c), indicating that IMQ induced distinct gene expression changes in control and mutant suprabasal cells. We also noted that IMQ-treated Ovol1−/− skin contained a higher number of epithelial cells that expressed both Krt1 and Krt14 than the other conditions, a finding validated by immunofluorescence detecting appreciable presence of keratin 14/keratin 14+ cells in intact Ovol1−/− skin (Figure 5d; Supplementary Figure S3d). Top marker genes of the IMQ-treated Ovol1−/− suprabasal cells included proinflammatory genes S100a8 and S100a9 (Ryckman et al., 2003; Wang et al., 2018) and keratin-encoding genes Krt6a, Krt6b, and Krt16 that have been shown to be upregulated in hyperproliferative or injured epidermis (Chen et al., 2019; Esaki et al., 2015; Lessard et al., 2013) (Figure 5e; Supplementary Table S4). Notably, the same S100a and keratin genes have been shown by scRNA-seq to be upregulated in the suprabasal cells of human psoriatic skin (Cheng et al., 2018). Epidermal basal cell clusters (Krt14high/Krt17+ /CD34−/Sostdc1−) were also computationally isolated and aggregated together for analysis (Supplementary Figure S3b and e). Again, IMQ elicited distinct gene expression changes in Ovol1−/− basal cells and control littermate counterparts, featuring upregulation of inflammation- and hyperproliferation-associated genes, including S100a8, S100a9, and Krt6a in the former (Supplementary Figure S3e and f; Supplementary Table S5).

We next analyzed the epidermal expression of select gene signatures associated with proliferation, differentiation, metabolism, and inflammation (Supplementary Table S6). Specifically, G2/M cell cycle checkpoint genes were enriched in basal cells, whereas the epidermal differentiation process was enriched in suprabasal cells, of the IMQ-treated Ovol1−/− skin (Figure 5f; Supplementary Figure S3g). Of note, expression of the early differentiation gene Kitdap was slightly upregulated in basal cells of untreated, but dramatically upregulated in basal cells of IMQ-treated, Ovol1−/− skin. Genes involved in metabolic processes (glycolysis, oxidative phosphorylation) were considerably more active in IMQ-treated Ovol1−/− epidermal cells in both basal and suprabasal compartments. IMQ-treated Ovol1−/− skin scored the highest for an inflammatory response—associated gene set in their suprabasal spinous cells, whereas IMQ-treated control skin scored the highest for this gene set in their basal keratinocytes, raising the possibility that Ovol1 deletion switches the major site of KC inflammatory response from basal cells to spinous cells. Overall, the molecular changes revealed by scRNA-seq analysis suggest significantly elevated rates of cellular flux characterized by increased basal cell proliferation and early differentiation, inefficient basal-to-mature spinous transition, and enhanced metabolism- and inflammation-associated gene expression in IMQ-treated Ovol1−/− skin epidermis.

To further investigate how Ovol1 loss impacts IMQ-induced changes in skin, fibroblasts from the four conditions were computationally aggregated and analyzed (Supplementary Figure S3b). A total of four clusters were observed: clusters 0 and 3 contained fibroblasts from untreated control and Ovol1−/− skin, whereas clusters 2 and 1 were enriched with fibroblasts from IMQ-treated control and Ovol1−/− skin, respectively (Supplementary Figure S3h). Top markers in IMQ-treated Ovol1−/− fibroblasts included inflammation-associated genes such as Saa3 and monocyte chemoattractant Ccl2 (Kratofil et al., 2017; Sack, 2018) (Supplementary Figure S3i; Supplementary Table S7). Moreover, IMQ induced a slight increase in the inflammatory response gene set score in control fibroblasts but a dramatic increase in Ovol1−/− fibroblasts (Supplementary Figure S3j).

Few immune cells were recovered in our 24-hour post-IMQ scRNA-seq analysis (Figure 5a and b). However, when these cells were computationally aggregated for analysis, it was still apparent that IMQ induced distinct gene expression changes in immune cells from Ovol1−/− and control littermate skin (Supplementary Figures S3b and S4a). CD3+ T cells in IMQ-treated Ovol1−/− skin showed elevated expression of Il17a and Il17f compared with counterparts from the other three conditions (Supplementary Figure S4b–d; Supplementary Table S8), suggesting a heightened T helper type 17 response in the Ovol1 mutant. Together, these findings show that Ovol1 deficiency results in an overall more inflammatory skin microenvironment affecting the responses of not only epidermal cells but also fibroblasts and immune cells.

**Inhibiting IL-1z signaling suppresses IMQ-induced skin inflammation in Ovol1-deficient mice**

IL-1 signaling represents a key proinflammatory pathway in myriad skin inflammatory processes (Cai et al., 2019; Murphy et al., 2000). The scRNA-seq data enabled us to specifically assess IL-1 pathway activity through examining the expression of ligands (Il1a, Il1b) and receptors (Il1r1, Il1r2) in different skin cell types. Expression of Il1a was barely detectable in the single-cell dataset, but Il1b expression was seen in the non-T immune cell cluster that are likely resident macrophage and dendritic cells (Supplementary Figure S5a). Il1r1 was expressed in most of the cell types with a slight fibroblast enrichment, whereas Il1r2 expression was highly enriched in the fibroblasts (Supplementary Figure S5a and b). Il1r2 expression responded differently to IMQ treatment in control and Ovol1−/− skin, characterized by increased expression in control epidermal basal and suprabasal cells and fibroblasts but decreased expression in Ovol1−/− epidermal cells and no detectable change in Ovol1−/− fibroblasts. In contrast, changes in Il1r1 expression were nonremarkable (Supplementary Figure S5b). Additionally, IMQ induced overall similar changes in the expression of Il1b and Il1r2 in immune cells of control and Ovol1−/− mice (Supplementary Figure S5c). Because Il1r2 encodes an IL-1 decoy receptor that inhibits IL-1β signaling (Schlüter et al., 2018), our findings suggest that the absence of Ovol1 results in a skin microenvironment that is no longer able to efficiently suppress IL-1 signaling when chemically challenged.

Despite the inability of scRNA-seq to detect Il1a transcripts, RNAscope analysis confirmed the bulk RNA-seq—revealed upregulation of Il1a mRNA in granular cells of Ovol1−/−, but not control, skin at 6 hours after IMQ treatment (Figure 6a). Furthermore, IL-1z protein was detected at greatly enhanced levels in loricrin-positive epidermal
granular cells of Ovol1−/− skin compared with control skin at 24 hours after IMQ treatment (Figure 6b and c). Western blotting showed that the full-length IL-1α precursor protein, but not the mature form, was predominantly elevated (Figure 6d). These results identify epidermal granular cells as the major source of IL-1α in inflamed Ovol1-deficient skin.

IL-1α is a dual function cytokine; its secreted and membrane-bound forms signal through the IL-1R1 (Il1r1) receptor, and its N-terminal calpain cleavage product translocates into the nucleus to regulate chromatin structure and transcription (Rider et al., 2013; Werman et al., 2004). To ask whether the excessively produced, full-length IL-1α in IMQ-treated Ovol1−/− skin epidermis acts through signaling, we pharmacologically inhibited the IL-1 signaling pathway. Specifically, we administered recombinant IL-1Ra, an antagonist of IL-1α/β through competitive receptor binding...
We have previously shown that Ovol1 is required for efficient cell cycle exit of embryonic epidermal progenitor cells (Nair et al., 2006). Ovol1 may function in a similar, cell-autonomous manner in IMQ-treated skin to help prevent excessive epidermal proliferation. Additionally, or alternatively, altered KC–immune cell cross-talk may contribute to the aggravated epidermal hyperplasia in IMQ-treated Ovol1-deficient mice through feedback mechanisms. Ovol1-deficient epidermal cells in IMQ-treated mice show an expansion of the Krt14/Krt1 double-positive subpopulation and molecular features of increased G2/M cell cycle transition, differentiation, and metabolism, suggesting that loss of Ovol1 in an inflammatory or stressed environment traps the KC in a state between active cycling and terminal differentiation.

The phenotypic rescue of Ovol1 mutant mice by IL-1Ra administration supports the functional importance of excessive full-length IL-1α signaling in mediating the aggravated skin inflammation and epidermal hyperplasia in these mice. Moreover, the finding that topical application of a recombinant IL-1Ra protein can be effective at symptom relief raises the possibility of such use in disease treatment. Given the association of OVOL1 SNPs with multiple inflammatory skin diseases, our study lays the groundwork for future investigation into the functional contribution of OVOL1 to disease pathogenesis and the underlying molecular mechanisms.

**MATERIALS AND METHODS**

**Mice**

Ovol1-LacZ (Ovol1tm1a(KOMP)Wtsi) mice in a C57BL/6N background, where a cassette composed of an Frt site, a LacZ sequence, and loxP sites is inserted into the Ovol1 locus, were purchased from UC Davis KOMP Repository (https://www.komp.org/). Ovol1+/− mice in a C57BL/6 strain background (Dai et al., 1998; Nair et al., 2006) were outcrossed onto a CD1 strain background and then intercrossed to produce homozygous mutant (Ovol1−/−) progeny for study. CD1 Ovol1−/− mice survive to adulthood but are sometimes smaller than control littersmates, so sex- and weight-matched control and mutant littersmates were used for all analyses. Information for all genotyping primers and control littermate genotypes used in each analysis is provided in Supplementary Tables S9 and S10. All animal studies have been approved and abide by regulatory guidelines of the Institutional Animal Care and Use Committee of the University of California, Irvine.

**IMQ-induced psoriasis model**

Mice at 7−8 weeks of age received a daily topical dose of 62.5 mg 5% IMQ cream (Perrigo, Allegan, MI) on shaved backs for six consecutive days or as indicated. Based on a previously described objective scoring system called PASI (van der Fits et al., 2009), erythema (redness of skin) and scaling (approximated by dry, white cracks and patches on skin surface) were blindly scored independently by one or more investigators, on a score from 0 (none) to 4 (most severe). The cumulative score (erythema plus scaling) served as a measure of the severity of clinical signs (score, 0–8).

**Flow cytometry**

To obtain single-cell suspensions, minced samples were digested with 10 ml of a solution containing 0.25% collagenase (Sigma, St. Louis, MO; C9091), 0.01 M HEPES (Thermo Fisher Scientific, Waltham, MA; BP310), 0.001 M sodium pyruvate (Thermo Fisher Scientific; BP356), and 0.1 mg/ml DNase (Sigma; DN25) at 37 °C for 1
hour with rotation, and then filtered through a 70-μm filter, spun down, and resuspended in 2% fetal bovine serum. A total of $5 \times 10^5$ cells were stained by incubation for 30 minutes at room temperature with the following antibodies diluted in PBS/2% fetal bovine serum: Alexa Fluor 488-conjugated anti-CD11b (BioLegend, San Diego, CA; 101217), phycoerythrin-conjugated anti-F4/80 (BioLegend; 123110), allopocynin-conjugated anti-CD45 (Tonbo Biosciences, San Diego, CA; clone 30-F11, 20-0451), allopocynin-Cy7-conjugated anti-Ly6G (Tonbo Biosciences; clone 1A8, 25-1276), 7-aminoactinomycin (BD Biosciences, San Jose, CA; 559925), and phycoerythrin-conjugated anti-CD49f (BD Biosciences; 555736).

**Bulk RNA-Seq and scRNA-Seq**

These experiments were performed as previously described (Haensel et al., 2020; Lee et al., 2014). For scRNA-seq, single cells were isolated from normal and inflamed back skin of adult mice, and live cells were FACs-sorted for analysis. Additional details are described in the Supplementary Materials and Methods.

**In vivo administration of recombinant IL-1Ra protein**

Purified IL-1Ra recombinant protein was produced as previously described (Zhang et al., 2009) and was dissolved in sterilized water to a concentration of 15 mg/ml. Same-sex/same-weight Ovol1−/− littermates received topical applications of IL-1Ra (40 mg/kg) or PBS on shaved back skin once at 30 minutes before IMQ treatment and then once every 24 hours until the end of experiments. Skin samples were fixed in 4% paraformaldehyde for H&E staining or embedded in OCT and frozen for immunostaining analysis.

**Statistics and reproducibility**

Most experiments were performed on at least three biological replicates or repeated at least twice. The sample size and number of independent experiments are indicated in the relevant figure legends. Disease pathology scoring was performed blindly. No data were excluded. For analysis of differences between groups, Student’s unpaired t-test was performed with 2-tailed in Excel. P-values of 0.05 or less were considered statistically significant. Error bars represent mean ± SEM. For bulk RNA-seq analysis, genes with a log2 fold change ≥1 and an adjusted P-value < 0.05 (generated by the negative binomial generalized models and Wald test employed by DESeq2) were considered significant and used to generate GO terms.

Additional details for these methods, as well as methods for β-galactosidase assay, human foreskin KC culture and RT-PCR, histology, immunostaining, western blotting, and RNAscope are described in Supplemental Materials and Methods.

**Data availability statement**

Datasets related to this article are deposited in the Gene Expression Omnibus database under accession code GSE158112. Code for single-cell analysis will be provided on request.

**ORCIDs**

- Peng Sun: http://orcid.org/0000-0001-9858-6537
- Remy Vu: http://orcid.org/0000-0002-1281-0728
- Morgan Dragan: http://orcid.org/0000-0002-6401-6816
- Daniel Haensel: http://orcid.org/0000-0001-5168-4068
- Guadalupe Gutierrez: http://orcid.org/0000-0003-1012-3664
- Quy Nguyen: http://orcid.org/0000-0002-7016-1155
- Elyse Greenberg: http://orcid.org/0000-0001-7171-7007
- Zeyu Chen: http://orcid.org/0000-0001-6393-1986
- Jie Wu: http://orcid.org/0000-0003-2759-5879
- Scott Atwood: http://orcid.org/0000-0001-7407-9792
- Eric Pearlman: http://orcid.org/0000-0003-0117-7582

Yuling Shi: http://orcid.org/0000-0002-1273-7881
Wei Han: http://orcid.org/0000-0002-2960-361X
Kai Kessenbrock: http://orcid.org/0000-0003-0410-6104
Xing Dai: http://orcid.org/0000-0001-8134-1365

**CONFLICT OF INTEREST**

The authors state no conflicts of interest.

**ACKNOWLEDGMENTS**

We thank Anand Ganesan and Sebastien de Feraudy for inspiration and advice, the Genomics High Throughput Facility and the Institute for Immunology FACS Core Facility at the University of California, Irvine (UCI) for expert service. This work was supported by National Institutes of Health (NIH) grants R01-AR068074 and R01-GM123731 (XD); RV, MD, and DH were partially supported by UCI National Science Foundation (NSF)-Simons Center for Multiscale Cell Fate Research through NSF grant DMS1562176 and Simons Foundation grant 594598 (QN). RV is a recipient of NSF predoctoral fellowship (DGE-1839285), and MD is partially supported by the NIH T32 Immunology Research Training Grant (AI 060573).

**AUTHOR CONTRIBUTIONS**

Conceptualization: XD; Data Curation: PS, RV, MD, DH, GG, QN, JW; Formal Analysis: PS, RV, MD, DH, GG, QN, JW; Funding Acquisition: XD; Investigation: PS, RV, MD, GG, ZC; Methodology: EG, EP, KK; Project Administration: XD; Resources: SA, YS, WH; Supervision: XD; Validation: PS, RV, MD, GG, ZC; Visualization: PS, RV, GG; Writing - Original Draft Preparation: XD; Writing - Review and Editing: XD, EP

**SUPPLEMENTARY MATERIAL**

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2020.10.025.

**REFERENCES**

Benhadou F, Minkoff D, Del Marmol V. Psoriasis: keratinocytes or immune cells - which is the trigger? Dermatology 2019;235:91–100.


