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Original artcle

GPR15 is not critically involved in the regulation of murine psoriasiform dermatitis

Tanya Sezin[1], Linda Kempen[1], Lisa-Maria Meyne, Sadegh Mousavi, Detlef Zillikens, Christian D. Sadik*

Department of Dermatology, Allergy, and Venereology, University of Lübeck, Lübeck, Germany

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ABSTRACT

Background: GPR15 has been implicated in the pathogenesis of T cell-driven inflammation of the skin and the gut. Expression levels of the GPR15 ligand GPR15 L are increased in psoriatic skin and considered as potential biomarker for the treatment response to anti-IL-17 antibody therapies. However, the significance of the GPR15 L/GPR15 for the pathogenesis of psoriasis and the mechanisms regulating GPR15 L expression are still elusive.
Objective: To determine the significance of GPR15 signaling in mouse models of psoriasis.
Methods: We addressed the role of the GPR15 L/GPR15 in the Aldara™-induced psoriasiform dermatitis (AIPD) and the IL-23-induced dermatitis model. In both models, we charted the expression levels of GPR15 L in the skin and assessed the significance of GPR15 L/GPR15 by examining *Gpr15*$^{-/-}$ mice.
Results: GPR15 L levels were increased in the AIPD, but not in the IL-23-induced dermatitis model. Deficiency in *Gpr15* did not alter the course of disease neither in the AIPD, nor in the IL-23-induced dermatitis model. In neither model, deficiency in *Gpr15* modulated disease on the histopathological or the molecular level. Despite the induction of GPR15 L in the AIPD model, GPR15^{+} cells did not accumulate in the skin.
Conclusion: GPR15 L expression is induced in psoriasiform dermatitis, but the activation of the IL-23/IL-17 axis alone is not sufficient for its induction. This restricts the potential use of GPR15 L levels as biomarker for the treatment response to anti-IL-17 antibody therapy. Our results leave a significant role of GPR15 in the pathogenesis of psoriasiform dermatitis rather unlikely. Hence, GPR15 L probably modulates psoriasiform dermatitis via GPR15-independent pathways.

1. Introduction

The G protein-coupled receptor 15 (GPR15) was first described in the mid-1990ies as a co-receptor for virus entry of the simian immunodeficiency virus (SIV) and the human immunodeficiency virus 2 (HIV-2) into immune cells [1]. GPR15 can be expressed on diverse cell types, including CD4^{+} and CD8^{+} T effector cells, T_{regs}, CD19^{+} B cells, dendritic epidermal T cells (DETC), neutrophils, monocytes/macrophages, and basal enterocytes [2–11].

More recently, GPR15 has been demonstrated to regulate homing of different T cell subsets to the gut and the skin [4–7 s. Thus, GPR15 modulates T cell recruitment and, consequently, the course of disease in several mouse models of colitis [2,3,12]. With respect to the role of GPR15 in the skin, it has been demonstrated that GPR15 regulates the recruitment of dendritic epidermal T cells (DETC) into the skin during embryogenesis. DETC seeding into the skin is delayed in *Gpr15*$^{-/-}$ mice but reaches normal levels by adulthood [10].

The cognate ligand of GPR15 has long remained elusive. Only recently, two independent studies have identified a 9 kDa, cationic polypeptide, encoded in humans by the gene *C10ORF99* (chromosome 10 open reading frame 99) and in mice by the gene *2610528A11Rik*, as cognate ligand of GPR15. "GPR15L" has been proposed as new designation for this polypeptide and "*Gpr15l*" for its gene [13,14]. The polypeptide had previously been known under the aliases "CSBF", which is a Sushi Containing Domain-2 (SUSD2)-binding factor (CSBF) inhibiting the cell growth of intestinal epithelium tumor cell lines [15], and "AP57". Under the latter alias, GPR15 L was highlighted as a novel type of multifunctional

Abbreviations: AIPD, aldara™-induced psoriasiform dermatitis; GPR15, G protein-coupled receptor 15; GPR15L, G protein-coupled receptor 15 ligand; WT, wild-type; TLR, toll-like receptor; rmIL-23, recombinant murine IL-23.

* Corresponding author.
E-mail address: Christian.Sadik@uksh.de (C.D. Sadik).

[1] equal contribution.

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antimicrobial peptide and as a promoter of dermal angiogenesis [16,17].

GPR15 L is expressed in the epithelia of the colon, the skin, the eye, the tongue, and the cervix, suggesting that the GPR15 L/GPR15 ligand/receptor pair may protect tissue homeostasis at the body's interfaces [14], but the functional significance of GPR15 L for physiology and pathophysiology is still vastly unknown. Suply et al. [14] reported that in a murine skin allotransplantation model, the rejection of skin grafts taken from *Gpr15l*$^{-/-}$ mice is delayed and that the recruitment of inflammatory cells, particularly of CD8^{+} T cells, into *Gpr15l*$^{-/-}$ skin grafts is reduced [14]. This supports the notion that Gpr15 L/GPR15 may play a role in T cell-driven immune responses in the skin.

Several lines of evidence also suggest a role of GPR15 L in the pathogenesis of psoriasis. Thus, GPR15 L is upregulated on mRNA and protein level in psoriatic skin lesions, and *C10ORF99* is one of the most significantly differentially methylated loci in psoriatic skin [14,18–20]. Notably, the levels of GPR15 L mRNA in psoriatic skin decline under treatment with the anti-IL-17 antibody secukinumab. This decline correlates with the improvement of the *Psoriasis Activity and Severity Index* (PASI) [14]. This suggests that cutaneous GPR15 L levels may be regulated in an IL-17-dependent manner. Supply et al. [14] accordingly proposed that GPR15 L levels in the skin may be a potential predictive biomarker for the treatment response of psoriasis patients to anti-IL-17 antibody therapies.

Recently, the pathogenic significance of GPR15 L for psoriasiform dermatitis has been addressed in the Aldara™-induced psoriasiform dermatitis (AIPD) mouse model [18]. This study revealed that, in this model, GPR15 L levels are increased in the skin. Furthermore, local knockdown of GPR15 L by shRNA in the skin ameliorated psoriasiform dermatitis, providing evidence that GPR15 L may promote the progression of AIPD. However, the mode of action of GPR15 L in psoriasiform dermatitis is unknown, and also the mechanism, which regulates GPR15 L expression, is largely elusive. We have therefore addressed the role of GPR15 in two previously described mouse models of psoriasis, namely the Aldara™-induced psoriasiform dermatitis (AIPD) and the IL-23-induced dermatitis model [10–12].

2. Materials and methods

2.1. Mice and genotyping

129P2-Gpr15$^{tm1.1Litt/J}$ mice (stock number: 008769) on the *C57BL/6* background were purchased from *The Jackson Laboratory* (Bar Harbor, ME, USA). All experiments were conducted in *Gpr15*$^{-/-}$ mice and their wild-type littermates at the age of 8–16 weeks. Mice were bred in the animal facility of the University of Lübeck, (Lübeck, Schleswig-Holstein, Germany). All animal experiments had been approved by the local government. The mice were genotyped according to the protocol provided by the *Jackson Laboratory* with slight modifications. Briefly, DNA was isolated from ear punch biopsies digested in 200 μl 50 mM NaOH solution for 45 min at 95 °C. Samples were neutralized by addition of 20 μl 1 M Tris HCl pH 8. PCR was performed with Phire Hot Start II DNA polymerase (Thermo Fischer Scientific GmbH) according to manufacturer's protocol. All primers were purchased from Biomers.net (biomers.net GmbH). The following primers were used for genotyping: oIMR9208 AAGGCACTTACCAGATTCAGCG; oIMR209 AGACAGATGTGTAGGACAGGTGGG; oIMR9210 GAAGTTCACCTTGATGCCGTTC. The expected molecular sizes of the PCR products of the WT and mutant alleles were 139 bp and 534 bp, respectively.

2.2. Aldara™-induced psoriasiform dermatitis (AIPD)

To induce AIPD, a 2 x 3 cm area on the mouse back was shaved and depilated on day -2 of the experiment. Starting on day 0, 50 mg Aldara™ cream (Meda, Solnau, Sweden), containing 5% imiquimod, were applied daily on this area for six consecutive days. Skin inflammation was evaluated daily using a modified version of the *Psoriasis Activity and Severity Index* (PASI), as previously described [21]. Briefly, erythema, infiltration, and desquamation were each individually scored on a scale from 0 to 4 with 0, none; 1, minimal; 2, mild; 3, distinct; 4; severe. The scores of these individual aspects of dermatitis were summed up to a cumulative score from 0–12. Subsequently, the area under the curve (AUC) of the cumulative score over time was calculated individually for each mouse.

2.3. IL-23-induced dermatitis

To induce IL-23-induced dermatitis, 500 ng of murine recombinant IL-23 (eBioscience) were applied intradermally in a final volume of 20 μl into the left ear every other day until day 14. 0.1% (w/v) BSA in PBS pH 7.2 served as vehicle control and was injected intradermally into the right ear. To quantify the severity of skin inflammation, ear thickness was measured every other day using a micrometer (Mitutoyo Corporation). On day 16, the experiment was terminated, and ears were harvested for downstream analyses.

2.4. Histopathology

For histopathology, skin samples obtained on the final day of the experiments were fixed in 4% (w/v) buffered formalin and paraffin-embedded, 6-μm tissues sections were stained with hematoxylin and eosin (H&E).

2.5. Determination of epidermal thickness

To measure epidermal thickness, five parallel lines stretching from the epidermal surface to the dermal-epidermal junction were randomly overlaid on a 200x magnified H&E image that was obtained for every section. The mean of the length of three independent images per section was calculated to represent the epidermal thickness per mouse. Shaved normal murine back skin from *Gpr15*$^{-/-}$ and WT mice served as a negative control.

2.6. Ki-67, CD31, and CD3 staining

To evaluate epidermal proliferation and the extent of angiogenesis and T lymphocytes infiltration, skin samples were stained for Ki-67, CD31, or CD3, respectively, as previously described [13,14]. Briefly, 6-μm murine back skin paraffin sections were deparaffinised and rehydrated according to a standard protocol. Heat-induced antigen retrieval was performed by boiling the slides in a pressure cooker for 10 min in 10 mM citrate buffer pH 6. Thereafter, slides were gradually cooled to RT and sections were blocked with 5% (v/v) normal goat or donkey serum (diluted in 0.01 M PBS pH 7.2) for 1 h at RT. 0.05 mg/ml of primary rat anti-murine Ki-67 antibody (BioLegend), rabbit anti-murine CD31 (Abcam) or rat anti-murine CD3e antibody (Dianova) diluted in 5% (v/v) normal goat or donkey serum and were applied for 1.5 h at RT. Following three washes with 0.01 M PBS, sections were incubated with 0.0055 mg/ml of biotin-SP-conjugated anti-rat IgG or Alexa-Fluor 594-conjugated goat anti-rat IgG (Jackson Immunoresearch) or with 0.0055 mg/ml AlexFluor 594-conjugated donkey anti-rabbit IgG (Jackson Immunoresearch) for CD31 staining for 1 h at room temperature. For Ki-67 staining, slides were washed and incubated with 0.002 mg/ml streptavidin-conjugated to DyLight 594 (Thermo Fischer Scientific) for 30 min at RT. After washing, slides were mounted with DAPI fluoromount G (SouthernBiotech). Total rat IgG or rabbit IgG were used as isotype controls. Slides were visualized and processed with BZ-9000E series Keyence

microscope and BZ-II analyzer program, respectively. To quantify epidermal hyperplasia, angiogenesis, and T cells infiltration in the skin, the number of Ki-67$^+$ cells, CD31$^+$ blood vessels, and CD3$^+$ cells was determined in three independent 200x magnification fields in each mouse.

2.7. Isolation of RNA and qPCR

Total RNA was extracted from snap frozen skin samples using TRIzol™ reagent (Invitrogen) following the manufacturer's instructions. RNA concentrations were determined by a Nanodrop 2000c spectrophotometer (Thermo Fischer Scientific GmbH). 500 ng of total RNA were transcribed using the ReverseAid First Strand cDNA Synthesis Kit (Thermo Fischer Scientific GmbH). Afterwards, qPCR, was performed using the SYBR Select Master Mix (Thermo Fischer Scientific GmbH) according to the manufacturers' instructions. All primers for qPCR were purchased from biomers.net (biomers.net GmbH) and are listed in Supplementary Table 1. qPCR was performed on the Eppendorf Mastercycler ep Realplex using the following cycling conditions: 50 °C for 2 min, 95 °C for 2 min, followed by 40 cycles each of 95 °C for 15 s, and 60 °C for 1 min each. The expression level of the gene of interest was normalized to the mRNA expression level of the *Gapdh* gene.

2.8. Co-culturing and stimulation of primary murine keratinocytes (KCs) and bone marrow-derived dendritic cells (BMDCs)

Primary murine keratinocytes (KCs) were isolated from adult tail skin of 8–12 weeks old *C57Bl6/J* mice, as previously described [22], resuspended in a density of 10^5 cells/ml in Keratinocyte growth medium (KGM) (PromoCell GmbH) supplemented with 1% (v/v) Pen/Strep, 10^5 cells were seeded into collagen-coated 24-well plates, and left for rest for 5 days. In parallel, BMDCs were isolated from the bone marrow of 8–12 weeks old *C57Bl6/J* mice, as previously described [23], and cultured in RPMI medium (Thermo Fischer Scientific GmbH) supplemented with 10% (v/v) FCS (Thermo Fischer Scientific GmbH), 1% (v/v) Pen/Strep, and 20 ng/ml of recombinant murine GM-CSF (PeproTech). The medium was replaced after 3 days. After 7 days, cells were spun down, washed with Ca^{2+}/Mg^{+2}-free HBSS pH 7.2, resuspended in KGM at a density of 10^6 cells/ml, before 25×10^4 BMDCs were added to resting KCs. These co-cultures were stimulated with a final concentration of 100 μg/ml isostearic acid (Sigma-Aldrich GmbH) and/or 10 μg/ml imiquimod (InvivoGen) complexed with the transfection reagent Lipofectamine 2000 (Thermo Fischer Scientific GmbH). For complexation, 2 μl Lipofectamine had first been mixed into 50 μl of KGM and had then been added to the equivalent volume of KGM medium containing imiquimod, isostearic acid, or both for 20 min at RT before added to the co-cultures. After 24 h, co-cultures cells were vigorously washed twice with Ca^{2+}/Mg^{2+}-free HBSS pH 7.2 to remove BDMCs, and total RNA was extracted from KCs. RNA was transcribed to cDNA, as described in Section 2.7, and expression levels of *Gpr15L*, *Cxcl1*, and *Cxcl2* were analyzed. All qPCR primers used in this study are listed in supplementary table S1.

2.9. Statistical analysis

All data are presented as mean ± SEM. Raw data were analyzed by Kruskal-Wallis test with Dunn's multiple-comparison test or by two-way ANOVA with Sidak's multiple-comparison test. $p < 0.05$ was considered statistically significant. All calculations were performed using GraphPad Prism 7.0 (GraphPad, San Diego, CA, USA).

3. Results

3.1. Significance of GPR15 in the AIPD model

To clarify the role of GPR15 signaling in the AIPD model, we compared the course of AIPD in *Gpr15*$^{-/-}$ mice and their wild-type (WT) littermates. Upon daily administration of 50 mg of Aldara™ cream onto the back skin, both WT and *Gpr15*$^{-/-}$ mice developed psoriasiform dermatitis, including erythema, infiltration of the skin, and desquamation (Fig. 1a). The severity of each of these features was scored, as described in *Methods*. WT and *Gpr15*$^{-/-}$ mice did not significantly differ in any of these features (Fig. 1b).

Consistent with the clinical score, lesional skin of WT and *Gpr15*$^{-/-}$ mice showed no histopathological difference in dermal infiltration by mononuclear cells and lymphocytes (Fig. 1c–e). WT and *Gpr15*$^{-/-}$ mice also displayed the same level of keratinocyte proliferation, quantified by the density of Ki-67$^+$ cells in the epidermis, and of epidermal hyperplasia (Fig. 2a–c). They also presented with the same level of angiogenesis in the dermis, as evaluated by the density of CD31$^+$ blood vessels in the dermis (Fig. 2d/e).

We assayed the mRNA expression levels of the cytokines involved in the regulation of AIPD, precisely of IL-23, IL-17A, IL-22, TNF-α, and IFN-γ, in the skin of WT and *Gpr15*$^{-/-}$ mice before the induction of AIPD and at the end of the experiment on day 6 by qPCR. There was no significant difference between WT and *Gpr15*$^{-/-}$ mice. In both, levels of IL-23 (precisely *Il23a*) and IL-22 were significantly increased on day 6 compared to day 0 (Fig. 3a). In contrast, IFN-γ mRNA levels were decreased, and IL-17A and TNF-α levels tended to be decreased in both groups.

To detect the possible accumulation of GPR15$^+$ cells in the skin, we compared the expression levels of GPR15 in naïve skin and lesional skin of WT mice. Under both conditions, GPR15 mRNA was abundant in the skin. However, the levels between day 0 and day 6 did not significantly differ and even tended to be lower in lesional skin than naïve skin (Fig. 3b). In line with this observation, we could not detect GPR15 expression in the skin, neither by GPR15-EGFP reporter mice, nor by immunohistochemistry (results not shown).

3.2. Significance of GPR15 in the IL-23-induced dermatitis model

We also examined the significance of GPR15 in the IL-23-induced dermatitis model. Like the AIPD model, this model recapitulates key features of human plaque psoriasis. However, in contrast to the AIPD model, the IL-23-induced dermatitis is solely driven by IL-23 and its major downstream effectors IL-17A and IL-22 [24,25].

IL-23-induced dermatitis was induced in WT and *Gpr15*$^{-/-}$ mice, as described in *Methods*. Its severity was assessed by determining the course of ear swelling over time. IL-23 elicited psoriasiform dermatitis in both WT and *Gpr15*$^{-/-}$ mice. There was no significant difference in disease severity between the two mouse strains, as evaluated by ear swelling and by histopathology (Fig. 4a). As previously described, on the histopathological level, IL-23 treatment caused a marked infiltration of the dermis with CD3$^+$ cells, which did not differ between the two groups (Fig. 4b). It also induced hyperproliferation of the epidermis, which we quantified by measuring epidermal thickness and by determining the density of Ki-67 expression in the epidermis. Both parameters for epidermal hyperproliferation were equal in WT and *Gpr15*$^{-/-}$ mice (Fig. 4c–e). The induction of T lymphocyte infiltration as well as epidermal hyperplasia were independent of the presence or absence of GPR15 (Fig. 4f).

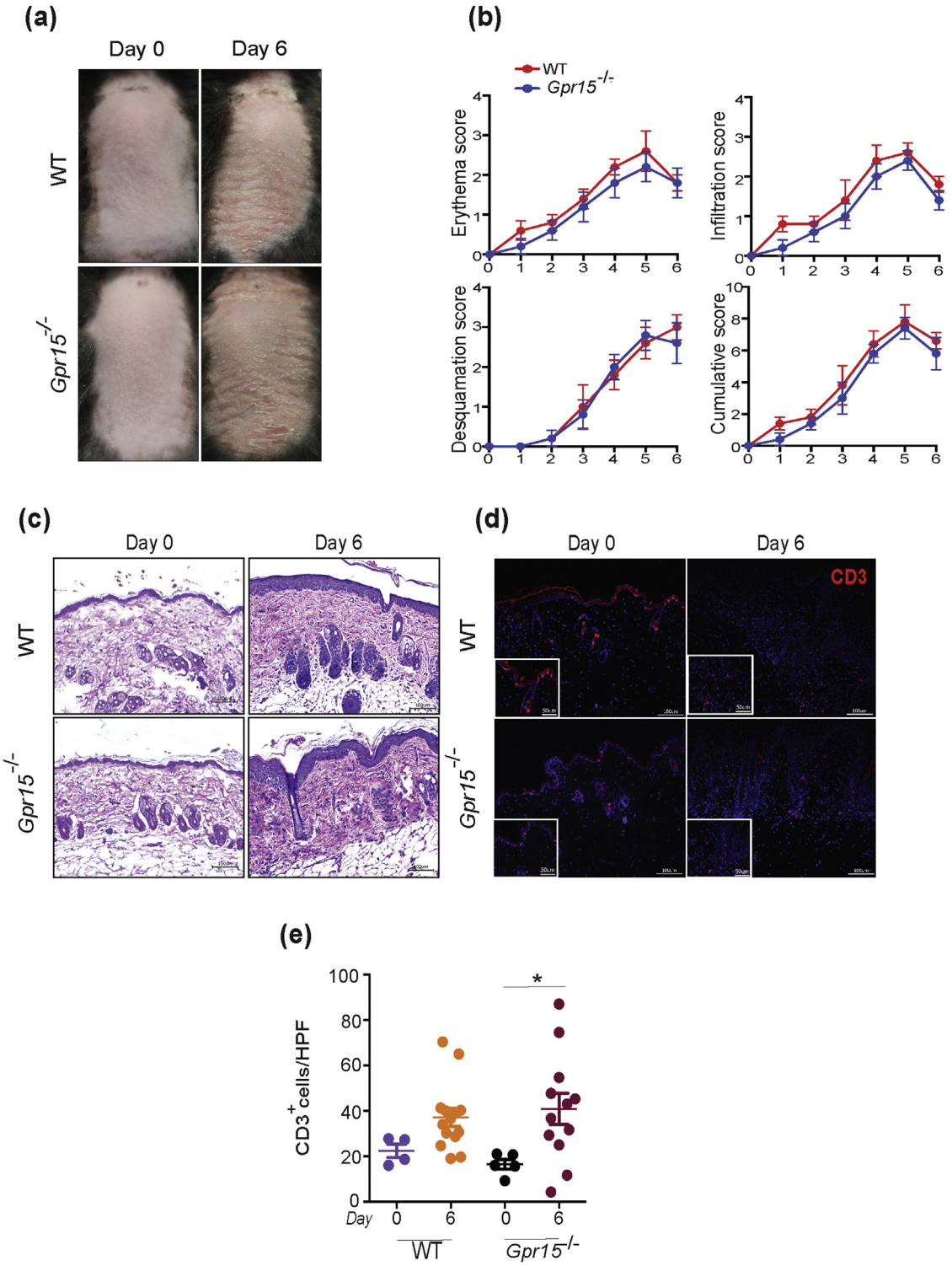

Fig. 1. *Gpr15* deficiency does not alter the course of disease in the AIPD model. (**a**) Representative clinical pictures of WT and *Gpr15*$^{-/-}$ mice before and 6 days after induction of AIPD. (**b**) The severity of the disease was evaluated based on erythema, infiltration, and desquamation, and the resulting cumulative score. One representative of three independent experiments is shown (n = 5 mice/group). GPR15 does not modify immune cell recruitment into the skin in the AIPD model. Representative pictures of (**c**) H&E- and (**d**) CD3-stained lesional skin of WT and *Gpr15*$^{-/-}$ mice on day 6 of the AIPD model. (**e**) Number of CD3^{+} cells/HPF in naïve and lesional skin of WT and *Gpr15*$^{-/-}$ mice on day 6. Data were pooled from three independent experiments. All data are presented as mean ± SEM. Results in (b) were analyzed for statistical significance by two-way ANOVA with Sidak's posthoc test. Results in (e) were tested for statistical significance by Kruskal Wallis test with Dunn's posthoc test; *, $p < 0.05$; scale bar: 100 μm.

3.3. GPR15 L is induced in the skin in the AIPD, but not in the IL-23-induced dermatitis model

We determined mRNA expression levels of the GPR15 ligand GPR15 L in the skin of WT and *Gpr15*$^{-/-}$ mice in both the AIPD and the IL-23-induced dermatitis model. In the AIPD model, we compared GPR15 L expression before the induction of disease (day 0) and on day 6 after induction of psoriasiform dermatitis (Fig. 5a). In naïve skin of both WT and *Gpr15*$^{-/-}$ mice, GPR15 L was only expressed in low levels. Treatment with Aldara™ induced GPR15 L

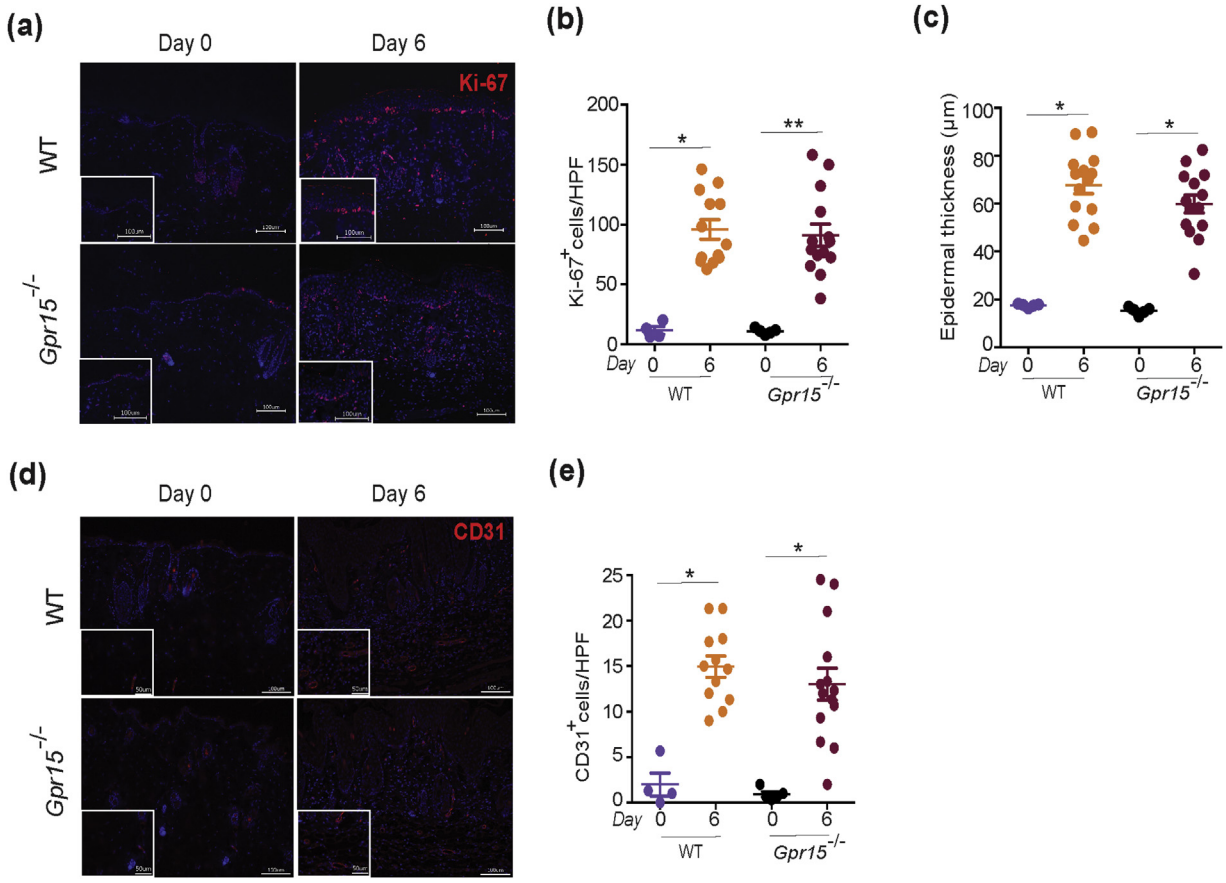

Fig. 2. GPR15 does not regulate keratinocyte hyperproliferation or angiogenesis in the AIPD model. (**a**) Representative pictures of Ki-67-stained skin sections, (**b**) number of Ki-67$^+$ cells/HPF in the epidermis, and (**c**) epidermal thickness (μm) in WT and *Gpr15*$^{-/-}$ mice on days 0 and 6. (**d**) Representative pictures of CD31 stainings and (**e**) and number of CD31$^+$ cells/HPF in the dermis. Data were pooled from three independent experiments and are presented as mean ± SEM. Results were tested for statistical significance by Kruskal Wallis test with Dunn's posthoc test. *, $p < 0.05$. **, $p < 0.01$; scale bar: 100 μm.

expression by approximately the 33-fold in WT and by the 48-fold *Gpr15*$^{-/-}$ mice by day 6 of the experiment.

We therefore addressed whether imiquimod and isostearic acid, the two pharmacologically active compounds of Aldara™ cooperatively driving the development of psoriasiform dermatitis [26], induce GPR15 L expression in keratinocytes, using a co-culture system of murine primary keratinocytes and BMDCs. Stimulation with imiquimod and/or isostearic acid, however, did not change GPR15 L expression (Fig. 5b), while upregulating the expression levels of the proinflammatory chemokines Cxcl1 and Cxcl2, used here a control for the principle responsiveness of keratinocytes to imiquimod and isostearic acid in our experimental setup (supplementary figure S1). Collectively, this indicates that the upregulation of GPR15 L in the AIPD model is not due to a direct effect of imiquimod and/or isostearic acid on keratinocytes and probably requires the activity of immune cell lineages other than DCs.

We next examined cutaneous GPR15 L expression in the IL-23-induced dermatitis model, comparing GPR15 L mRNA levels in naïve skin and in vehicle- and IL-23-treated skin. Neither in vehicle, nor in IL-23-treated skin, GPR15 L expression differed from naïve skin (Fig. 5c). This indicates that IL-23 alone is not sufficient to induce GPR15 L. Furthermore, this result excludes that mechanical manipulation of the skin, as it already occurs by regular injection of the skin with vehicle, is sufficient to affect GPR15 L expression levels.

4. Discussion

In this study, we have examined the role of GPR15 in psoriasis, using the AIPD and the IL-23-induced dermatitis mouse model. Both models mimic hallmarks of psoriasis on the clinical, the histopathological, and the molecular level [24,27]. We have scrutinized the impact of *Gpr15* deficiency on all clinical and histopathological major hallmarks of psoriasis, including erythema, skin infiltration, and desquamation on the clinical level and immune cell recruitment, keratinocyte proliferation, and dermal angiogenesis on the histopathological level. *Gpr15* deficiency did not alter the progression of any of these hallmarks. This also included T cell recruitment into the skin. The lack of an effect of *Gpr15* deficiency on T cell recruitment is remarkable because GPR15 has been suggested to be important for T cell recruitment into the skin in the rejection of skin allotransplantats [14].

Our results in the AIPD model are intriguing particularly when considering that, while this manuscript was in preparation, deficiency in GPR15 L was reported to attenuate disease in the AIPD model [18]. Although Chen et al. [18] conducted their study in mice on the *Balb/c* background, while the mice in our study were on the *C57BL/6* background, thus, limiting the comparability of the results of two studies, in combination the two studies suggest that GPR15 L may exert its effects in the AIPD model through GPR15-independent mechanisms. This notion is further supported when considering that Chen et al. [18] concluded from in vitro

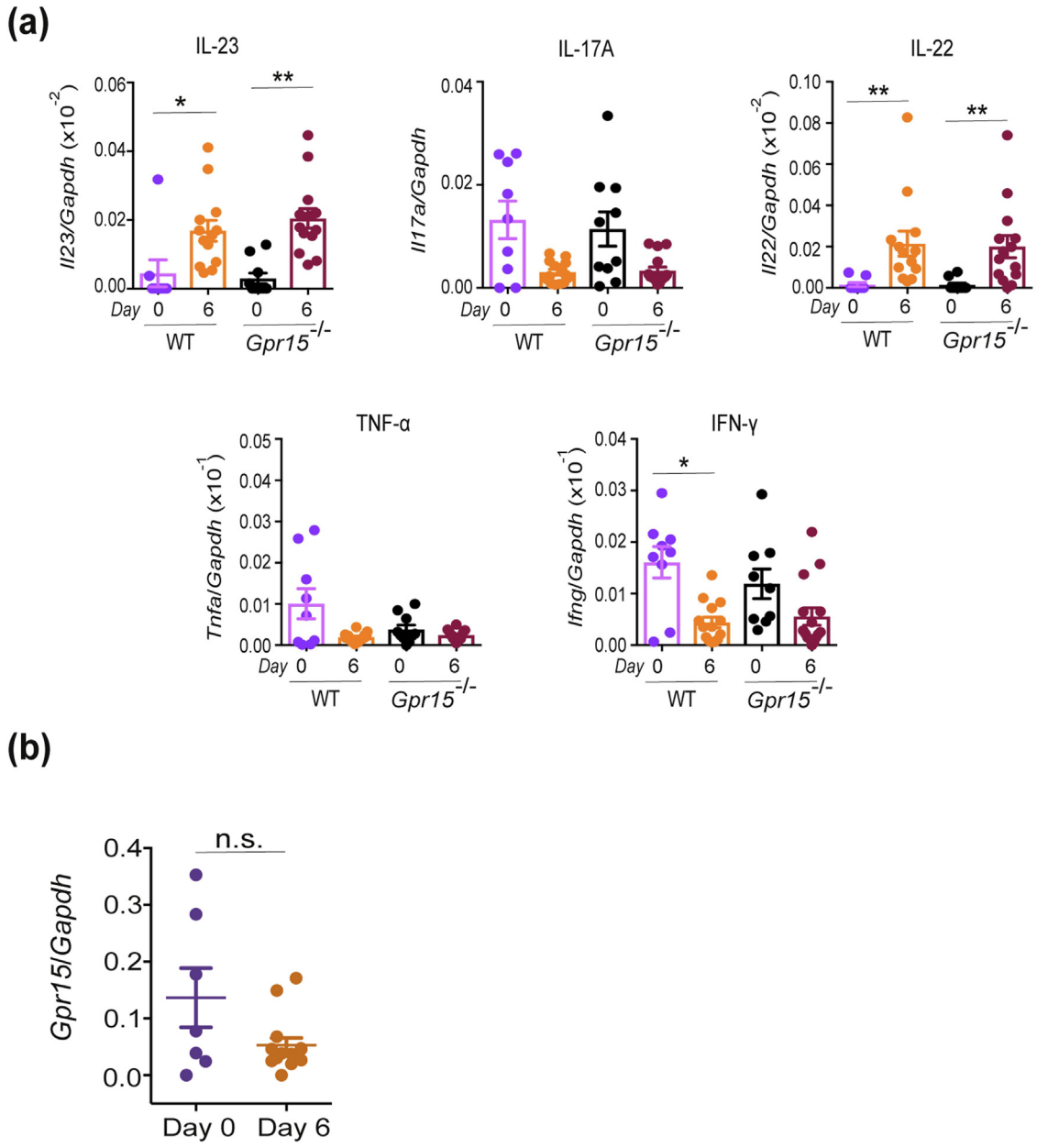

Fig. 3. Gene expression of psoriasis-related cytokines and *Gpr15* in the skin in the AIPD model. (**a**) mRNA expression levels in naïve (day 0) and Aldara™-treated (day 6) skin of WT and *Gpr15*$^{-/-}$ mice. (**b**) GPR15 expression is not changed in lesional skin in the AIPD model. *Gpr15* mRNA expression levels in naïve (day 0) and Aldara™-treated (day 6) skin of WT mice. All expression were normalized to *Gapdh* mRNA expression levels. Data were pooled from at least two independent experiments and are presented as mean ± SEM. Results were tested for statistical significance by Kruskal Wallis test with Dunn's posthoc test; *, $p < 0.05$; **, $p < 0.01$, n.s., not significant.

experiments in HaCaT cells that GPR15 L may aggravate AIPD by promoting keratinocyte proliferation. Expression of GPR15 in keratinocytes has not been reported, and, in this study, we did not detect GPR15 expression on keratinocytes either. Hence, GPR15 L likely promotes the proliferation of keratinocytes through mechanisms other than GPR15 signaling.

In line with two most recent reports [14,18], we have found that GPR15 L mRNA levels are elevated in the skin upon treatment with Aldara™. Thus, AIPD model mimics human plaque psoriasis also in this aspect. In contrast, GPR15 L was not upregulated in the IL-23-induced dermatitis model. This difference between the two models provides insights into the molecular mechanisms inducing GPR15 L in AIPD and human plaque psoriasis: first, as both the AIPD and IL-23-induced dermatitis model feature a marked proliferation of keratinocytes, the increased expression of GPR15 L cannot be simply due to more keratinocytes present in the skin. Second, the activation of IL-23 and its downstream pathways alone is not sufficient or does not participate at all in the induction of GPR15 L. This finding is of importance because the IL-23 pathway is not only critical for IL-23-induced dermatitis, but also for AIPD and human plaque psoriasis [27,28]. Moreover, the decline in GPR15 L levels in the skin in response to the IL-17A neutralizing antibody secukinumab correlates to the improvement of the PASI score [29]. As the induction of IL-17A in psoriasis is thought to be primarily controlled through IL-23 [29], our results suggest that the reduction in GPR15 L levels under treatment with secukinumab are not a direct result of the inhibition of IL-17A but rather a secondary effect. This must be considered when

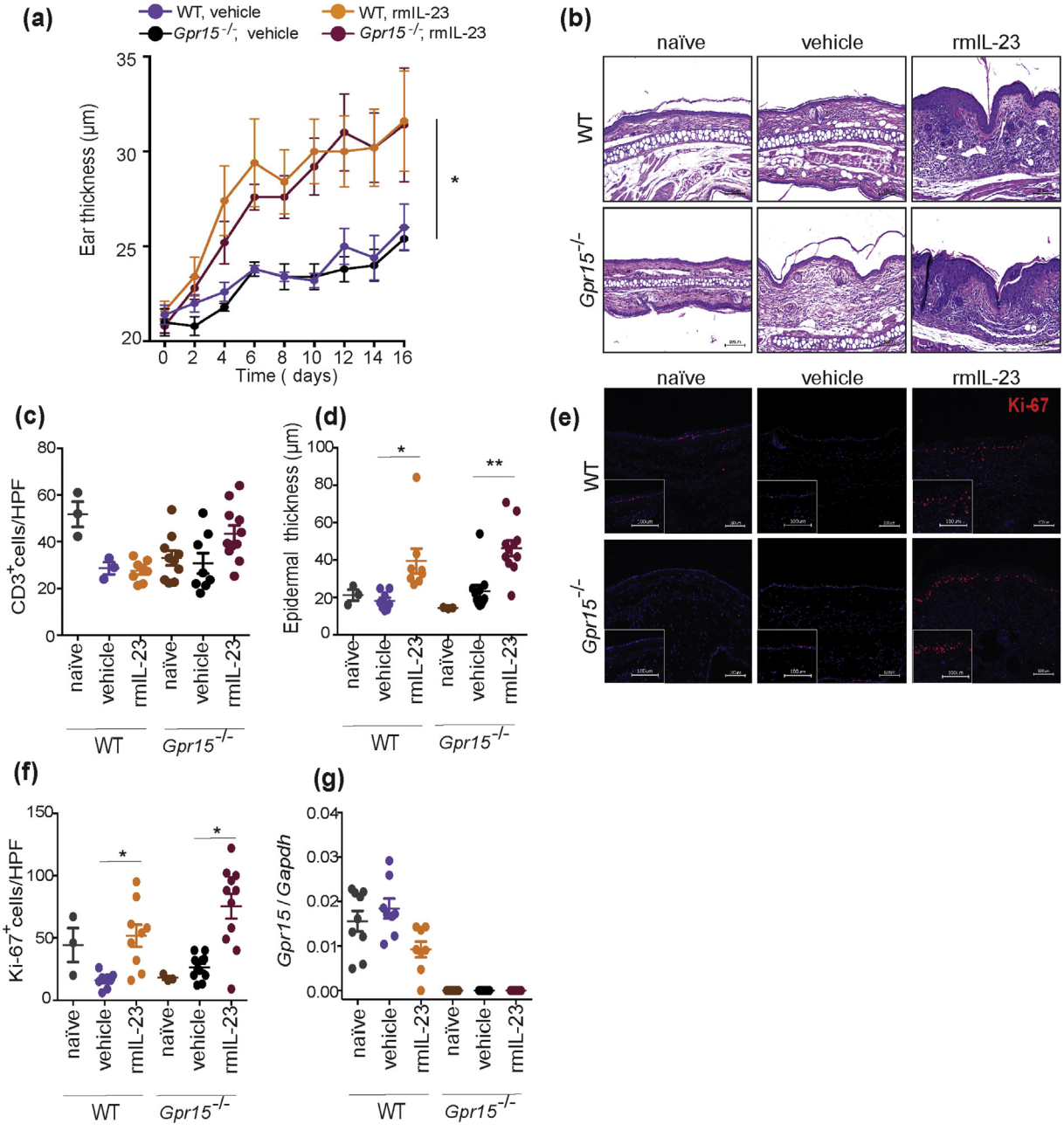

Fig. 4. GPR15 does not modulate IL-23-induced dermatitis. Comparison of naïve skin and vehicle- or IL-23-treated skin of WT and *Gpr15*$^{-/-}$ mice on day 16 of the IL-23-induced dermatitis model with (**a**) Ear thickness over time, (**b**) representative H&E stainings (**c**) CD3^{+} cells/HPF, (**d**) epidermal thickness, (**e**) representative Ki-67 stainings, (**f**) Ki-67^{+} cells/HPF in the epidermis, and (**g**) GPR15 mRNA expression in the skin. Data are presented as mean ± SEM. In (a one representative of two independent experiments is shown (n = 5 mice/group). In (c), (d), (f), and (g) data were pooled from two independent experiments. Results in (a) were tested for statistical significance by two-way ANOVA with Sidak's posthoc test, results in (c), (d), (f) and (g) by Kruskal Wallis test with Dunn's posthoc test. *, $p < 0.05$; **, $p < 0.01$. scale bar: 100 µm.

evaluating GPR15 L levels in the skin as biomarkers for the treatment response to anti-IL-17A antibodies, as suggested by Supply et al. [14].

GPR15 L is a multifunctional antimicrobial peptide active against certain gram-positive bacteria, including *Staphylococcus aureus*, viruses, and fungi [16]. Aldara™ induces psoriasiform dermatitis by activating TLR7 by imiquimod and the inflammasome in keratinocytes by isostearic acid [26]. Thus, Aldara™ induces psoriasiform dermatitis by activating the antimicrobial host defense in the skin. The antimicrobial host defense is also activated in psoriatic skin lesions, which is, among others, reflected by the upregulation of diverse antimicrobial proteins in psoriatic skin [30]. Herein, the activation of the host defense may be an instigator of psoriatic flares, a result of an unspecific activation of keratinocytes through the activated immune system, or the result of the comprised physical integrity of psoriatic skin, consequently, getting into contact with microbes.

In summary, our results argue against GPR15 as promising therapeutic target in psoriasis and warrant caution for the potential use of GPR15 L levels in the skin as biomarkers for the treatment response to anti-IL-17A antibodies in psoriasis. Future studies are required to delineate which receptor other than GPR15 mediates the described disease-promoting effect of GPR15 L in psoriasis.

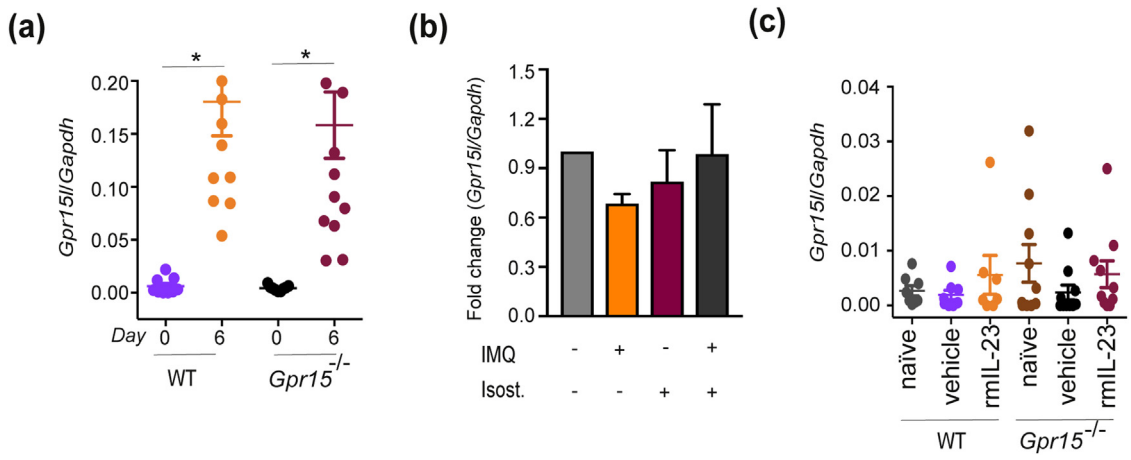

Fig. 5. GPR15 L is upregulated in murine models of psoriasis in an IL-23-independent manner. (**a**) Gene expression levels of GPR15 L in naïve (day 0) and Aldara™-treated skin (day 6) of WT and *Gpr15*$^{-/-}$ mice in the AIPD model. (**b**) GPR15 L gene expression levels relative to unstimulated control in murine primary keratinocytes after co-culture with bone marrow-derived DCs and stimulation with imiquimod and/or isostearic acid (Isost.). Results are compiled from 4 independent experiments. (**c**) Gene expression levels of GPR15 L in naïve skin and vehicle- or IL-23-treated skin on day 16 in WT and *Gpr15*$^{-/-}$ mice. Data were pooled from at least two independent experiments. Results are presented as mean ± SEM and were tested for statistical significance by Kruskal Wallis test with Dunn's posthoc test; *, $p < 0.05$.

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Conflict of interest

The authors have no conflict of interest to declare

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.01.008.

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