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RESEARCH ARTICLE

Keratinocytes activated by IL-4/IL-13 express IL-2Rγ with consequences on epidermal barrier function

Audrey Progneaux[1] | Céline Evrard[1] | Valérie De Glas[1] | Alix Fontaine[1] | Céline Dotreppe[1] | Evelyne De Vuyst[1] | Arjen F. Nikkels[2] | Vicente García-González[3] | Laure Dumoutier[4] | Catherine Lambert de Rouvroit[1] | Yves Poumay[1]

[1]Research Unit of Molecular Physiology (URPhyM), NAmur Research Institute for LIfe Sciences (NARILIS), University of Namur, Namur, Belgium

[2]Department of Dermatology, CHU of Sart Tilman, University of Liège, Liège, Belgium

[3]Almirall S.A., Barcelona, Spain

[4]Experimental Medicine Unit, De Duve Institute, Université catholique de Louvain, Brussels, Belgium

Correspondence
Yves Poumay, URPHYM-Department of Medicine, University of Namur, 61 Rue de Bruxelles, B-5000 Namur, Belgium.
Email: yves.poumay@unamur.be

Abstract

Atopic dermatitis (AD) is a Th2-type inflammatory disease characterized by an alteration of epidermal barrier following the release of IL-4 and IL-13. These cytokines activate type II IL-4Rα/IL-13Rα1 receptors in the keratinocyte. Whilst IL-2Rγ, that forms type I receptor for IL-4, is only expressed in haematopoietic cells, recent studies suggest its induction in keratinocytes, which questions about its role. We studied expression of IL-2Rγ in keratinocytes and its role in alteration of keratinocyte function and epidermal barrier. IL-2Rγ expression in keratinocytes was studied using both reconstructed human epidermis (RHE) exposed to IL-4/IL-13 and AD skin. IL-2Rγ induction by type II receptor has been analyzed using JAK inhibitors and RHE knockout (KO) for IL13RA1. IL-2Rγ function was investigated in RHE KO for IL2RG. In RHE, IL-4/IL-13 induce expression of IL-2Rγ at the mRNA and protein levels. Its mRNA expression is also visualized in keratinocytes of lesional AD skin. IL-2Rγ expression is low in RHE treated with JAK inhibitors and absent in RHE KO for IL13RA1. Exposure to IL-4/IL-13 alters epidermal barrier, but this alteration is absent in RHE KO for IL2RG. A more important induction of IL-13Rα2 is reported in RHE KO for IL2RG than in not edited RHE. These results demonstrate IL-2Rγ induction in keratinocytes through activation of type II receptor. IL-2Rγ is involved in the alteration of the epidermal barrier and in the regulation of IL-13Rα2 expression. Observation of IL-2Rγ expression by keratinocytes inside AD lesional skin suggests a role for this receptor subunit in the disease.

KEYWORDS
atopic dermatitis, barrier function, interleukins, keratinocyte biology, Signal Transduction.

1 | INTRODUCTION

Atopic dermatitis (AD) is a common inflammatory skin disease that affects the epidermal barrier through dysregulation of the cutaneous immune response. Th2 immune response results in the production and local release of cytokines and causes of further epidermal barrier alterations. Interleukin (IL)-4 and IL-13 released in AD skin bind to receptors on keratinocytes, impeding the correct efficiency and maintenance of the barrier.[1–5]

On keratinocytes, IL-4 and IL-13 bind to type II IL-4Rα/IL-13Rα1 receptor, activating the JAK/STAT, MAPK and PI3K/AKT pathways.[5–8] It is assumed that type I IL-2Rγ/IL-4Rα receptor, which only

Abbreviations: AD, atopic dermatitis; CA2, carbonic anhydrase 2; FLG, filaggrin; IL, interleukin; JAK–STAT, Janus kinase-signal transducer and activator of transcription signalling; LCA, leukocyte common antigen; LOR, loricrin; NELL2, neural epidermal growth factor-like 2; RHE, reconstructed human epidermis; TEER, transepithelial electrical resistance.

binds IL-4, is solely expressed by haematopoietic cells. The IL-13Rα2 subunit, binding IL-13 only but with higher affinity, is considered as decoy receptor, competing with IL-4Rα/IL-13Rα1 receptor to bond IL-13.[5,9–12]

Targeted therapies of AD include blocking antibodies directed against receptors or their ligands, or small molecules blocking downstream signalling pathways such as JAK inhibitors. Recently, treatment of moderate-to-severe AD relies on dupilumab, targeting IL-4Rα, but therapies targeting IL-13 are also efficient, preventing binding to both IL-13Rα1 and IL-13Rα2 by tralokinumab or the dimerization between IL-4Rα and IL-13Rα1 by lebrikizumab. The protection of epidermal barrier with these strategies is a strong indication that they interfere with IL-4 and IL-13 receptors and downstream signalling in keratinocytes. Heterogeneity of AD, in terms of clinical presentation and responses to therapies, highlights the need for further investigation of the pathological mechanisms that involve IL-4 and IL-13 receptors in keratinocytes.[13–18]

In vitro, reconstructed human epidermis (RHE) are obtained by culturing keratinocytes on a polycarbonate filter and positioning at air-liquid interface.[19] RHE exposed to IL-4 and IL-13 acquire some histological characteristics of AD, such as hypogranulosis, spongiosis and increased barrier permeability. Simultaneously, exposure of RHE to IL-4 and IL-13 dysregulates gene expression in keratinocytes, as observed in AD skin. For instance, expression of carbonic anhydrase II (CA2) and neural epidermal growth factor-like 2 (NELL2) becomes elevated, whilst loricrin (LOR) and filaggrin (FLG) expression is reduced.[20–22]

Since the RHE model lacks immune cells, it allows to dissect roles of cytokine receptor pathways in keratinocytes. Analysis of gene expression in RHE exposed to Th2 cytokines revealed significant upregulation of IL-2Rγ mRNA,[23] suggesting potential involvement of IL-2Rγ receptor subunit in AD keratinocytes.

Here we further characterize the induction of IL-2Rγ expression in RHE exposed to IL-4 and IL-13 and illustrate the presence in AD of IL-2Rγ mRNA in epidermal keratinocytes. Using JAK inhibitors and gene inactivation in RHE models, we report IL-2Rγ induction through activation of IL-4Rα/IL-13Rα1 receptor. Furthermore, we highlight the role of IL-2Rγ receptor subunit in keratinocytes, responsible for barrier alterations in response to Th2 cytokines.

2 | METHODS

2.1 | Origin of cells and tissues, culture of epidermal keratinocytes, and treatments

Skin biopsies (University Hospital in Liège, Belgium) from patients with AD were collected and stored at −70°C in OCT (Tissue-Tek, Sakura Finetek, California, USA). Healthy skin biopsies and normal keratinocytes were isolated from abdominoplasties (Clinique St Luc, Namur, Belgium) as described.[24] All samples were obtained after written informed consent. Studies were approved by local Ethical Committees, in accordance with the Declaration of Helsinki Principles. HEKa normal adult human keratinocytes were purchased from Thermo Fisher Scientific (Massachusetts, USA). Immortalized N/TERT keratinocytes came from J.G. Rheinwald's laboratory.[25] Cells authenticity was assessed at ATCC (Manassas, VA) (http://www.atcc.org) by comparison of an original frozen vial of cells[26] with current cultured N/TERT cells. Keratinocytes were cultured to confluence in Epilife medium (Thermo Fisher Scientific, Massachusetts, USA) supplemented with HKGS (Cascade Biologics, Portland, Oregon, USA), penicillin 50 U/mL and streptomycin 50 μg/mL (Sigma-Aldrich, Missouri, USA) or used to reconstruct human epidermis on polycarbonate filters during 11 days as described.[19,27] Keratinocytes or RHE were incubated with IL-4 (50 ng/mL) and IL-13 (50 ng/mL) (PreproTech, New Jersey, USA) for timings mentioned in the Results section. To mimic differentiation keratinocyte monolayers were cultured in medium with a 1.5 mM calcium ion concentration. RHE were pretreated overnight with JAK inhibitors tofacitinib (1 μM), upadacitinib (0.2 μM), ruxolitinib (1 μM) and deucravacitinib (1 μM) (Almirall, Barcelona, Spain).

2.2 | CRISPR-Cas9 inactivation of IL13RA1 or IL2RG in keratinocytes

IL13RA1 (cytogenetic location Xq24) and IL2RG genes (cytogenetic location Xq13.1) were each inactivated in immortalized N/TERT keratinocytes using the CRISPR-Cas9 method as described.[28] Oligonucleotide sequences and primers are respectively listed in Tables S1 and S2.

Clones exhibiting a deletion in IL13RA1 were screened for large genomic deletions spanning exons 2–11 by PCR. The mutations were characterized by Sanger sequencing of PCR products through Mix2Seq Kit (Eurofins, Gembloux, Belgium). Clones IL13RA1$^{UNΔ1/0}$ and IL13RA1$^{UNΔ2/0}$ were identified with such deletions, characterized at the nucleotide level by sequencing PCR products, and respectively named clones IL13RA1$^{-/0}$(a) and IL13RA1$^{-/0}$(b) (Figure S1). Considering +1 as the genomic position corresponding to A in the AUG initiation codon, allele UNΔ1 carries a deletion encompassing nucleotides +13 470 to +64 174, with a short open reading frame coding for 62 amino acid residues of IL-13Rα1 protein instead of the complete 428 residues sequence. Allele UNΔ2 exhibits a deletion of nucleotides from +13 452 to +64 175, encoding 98 amino acid residues only.

Clones exhibiting a deletion in IL2RG were screened for large genomic deletions spanning exons 2–8 by PCR. Edition of DNA was characterized by sequencing, and three clones IL2RG$^{UNΔ1/0}$, IL2RG$^{UNΔ2/0}$ and IL2RG$^{UNΔ3/0}$ were determined and named IL2RG$^{-/0}$(a), IL2RG$^{-/0}$(b) and IL2RG$^{-/0}$(c) (Figure S2). Considering +1 as the genomic position that corresponds to A in AUG initiation codon, allele UNΔ1 is IL2RG with a deletion between +591 and +3625 with a short open reading frame consisting of 127

amino acid residues of IL-2Rγ protein instead of the normal 370 amino acid sequence. Allele UNΔ2 exhibits a deletion between +624 and +3625 and encodes 138 amino acid residues, whereas UNΔ3 carries two deletions, one between +592 and +600, and the other between +624 and + 3625, coding for a 135 amino acid sequence.

2.3 | Statistical analysis

Data are presented as means ±SD and graphs are performed using GraphPad Prism 5 software. Statistics are calculated using software "SigmaPlot 14.0" using one-way ANOVA repeated measures. Values statistically significant are labelled */# where $p < 0.05$, **/## where $p < 0.005$, and ***/### where $p < 0.001$.

3 | RESULTS

3.1 | Expression of the IL-2Rγ receptor subunit is upregulated in vitro in RHE exposed to IL-4 and/or IL-13 and in vivo in AD lesional epidermis

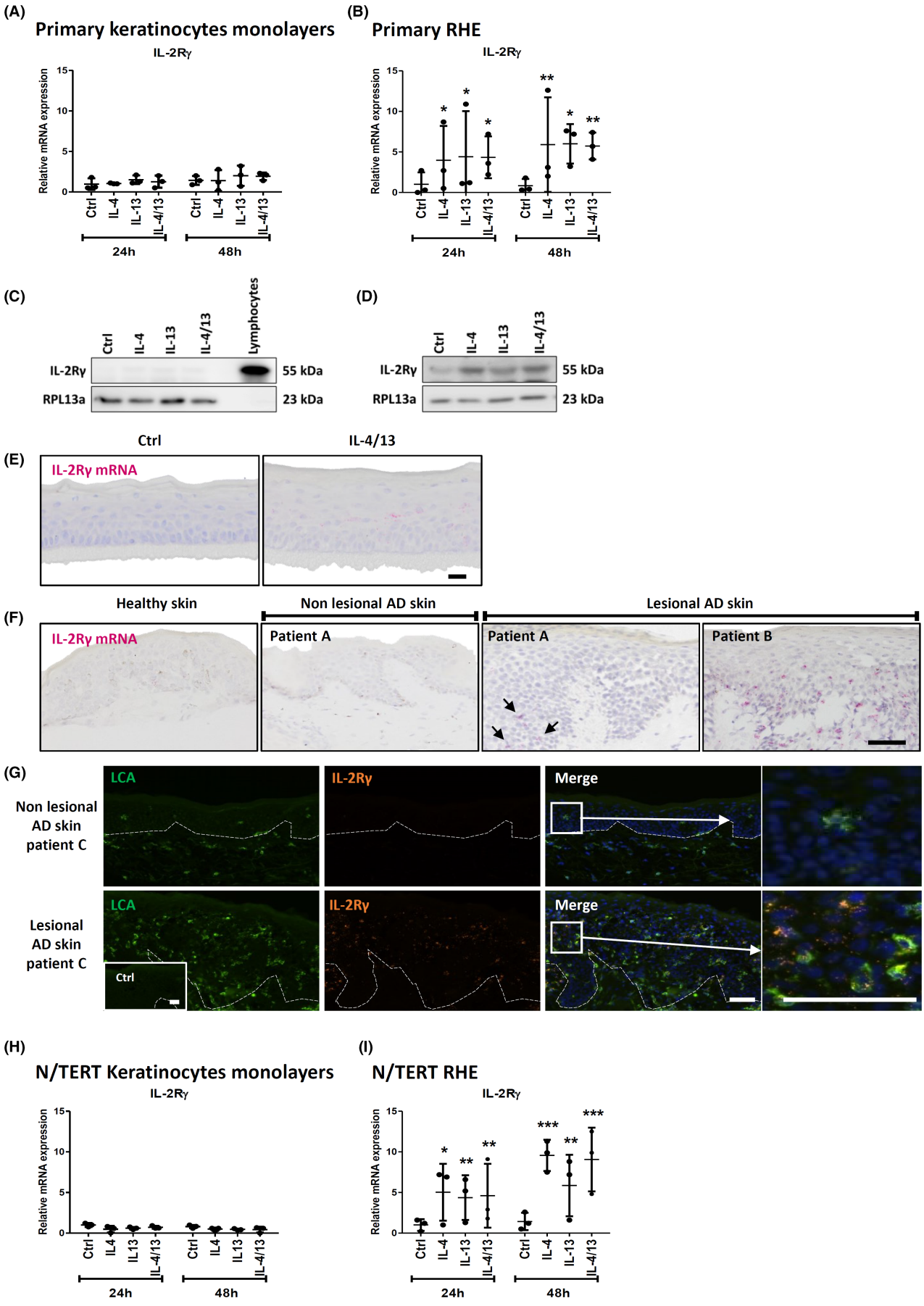
Expression of the IL-2Rγ receptor subunit is almost undetected in normal keratinocytes cultured as monolayers or in RHE. However, some slight but significant induction of IL-2Rγ expression occurs in RHE only, in response to exposure to IL-4 and/or IL-13 (Figure 1A–D). In consequence, IL-4Rα/IL-2Rγ receptor for IL-4 could organize and possibly function in epidermal keratinocytes challenged by Th2 interleukins. Expression data suggest that expression of IL-2Rγ might essentially occur in keratinocytes undergoing late differentiation.[24,29] Induction of IL-2Rγ expression is investigated in keratinocyte monolayers cultured in 1.5 mM calcium ion concentration to initiate differentiation as characterized by increased expression of markers like keratin 10, filaggrin and loricrin (Figure S3A). IL-2Rγ expression cannot be induced in differentiated keratinocytes by incubation with IL-4 and IL-13, whilst in epidermis reconstructed with same cells, exposure to IL-4 and IL-13 increases expression of IL-2Rγ (Figure S3B), strengthening data presented in Figure 1. These results confirm the requirement for a stratified epidermis to observe IL-2Rγ expression. Localization of IL-2Rγ mRNA expression was investigated using in situ hybridization in RHE sections and found in living cell layers of RHE exposed to IL-4 and IL-13 (Figure 1E). In AD skin, IL-2Rγ mRNA signal in epidermis is elevated in lesional areas, whilst scarcely observed in non-lesional areas and healthy skin (Figure 1F). To discriminate between immune cells and keratinocytes to localize IL-2Rγ expression, leukocyte common antigen (LCA) was labelled by immunofluorescence (Figure 1G). Although double-positive cells for LCA and IL-2Rγ-encoding mRNA are observed in lesional AD skin, the observation of cells solely labelled for IL-2Rγ-encoding mRNA strongly suggests that IL-2Rγ expression happens in keratinocytes.

3.2 | Enhanced IL-2Rγ expression in RHE results from activation by IL-4 and IL-13 of type II IL-4Rα/IL-13Rα1 receptor and downstream signalling

IL-4Rα/IL-13Rα1 receptor being the only receptor for IL-4 and IL-13 expressed in keratinocytes in healthy skin,[30] downstream signalling was probed in RHE after 15 min exposure to both ligands. As expected, enhanced STAT3 and STAT6 phosphorylation is observed in such conditions. STAT6 activation is inhibited by JAK inhibitors, strongly by tofacitinib, upadacitinib, or ruxolitinib, and partly by deucravacitinib. STAT3 activation is inhibited by the four inhibitors (Figure 2A). Interestingly, all of them significantly prevent the expression of IL-2Rγ induced by 48 h exposure to IL-4 and IL-13, indicating role of IL-4Rα/IL-13Rα1 receptor and JAK signalling in this induction (Figure 2B). Whilst tofacitinib and upadacitinib are found more potent than ruxolitinib and deucravacitinib at preventing the induction of IL-2Rγ expression, the efficiency of all inhibitors was verified through analysis of AD markers. Tofacitinib and upadacitinib decrease the mRNA expression of CA2 and NELL2 whilst ruxolitinib solely alters CA2 expression. All JAK inhibitors prevent decreased expression of FLG and LOR usually observed in RHE exposed to IL-4 and IL-13 (Figure S4).

The role played by IL-4Rα/IL-13Rα1 receptor activation in the phenotype alteration of keratinocytes exposed to IL-4 and IL-13 was confirmed by rendering keratinocytes deficient for the IL-13Rα1 receptor subunit. For this purpose, we chose the N/TERT keratinocyte cell line which allows tissue reconstruction.[25] Except morphological alterations such as spongiosis and hypogranulosis (Figure S5A), responses

FIGURE 1 IL-2Rγ is expressed in some keratinocytes of living layers of RHE challenged by IL-4 and IL-13 and in lesional AD epidermis. (A, B, H–I) Relative mRNA expression of IL-2Rγ in keratinocytes monolayers (A, H) and RHE (B, I) using primary (A, B) or N/TERT (H–I) keratinocytes exposed for 24 and 48 h to IL-4 and/or IL-13 (50 ng/mL), or not. Level of mRNA was assessed through RT-qPCR. RPLP0 was used as reference gene. Values are expressed relative to Ctrl 24 h (mean ± SD, $n = 3$, one-way ANOVA, * indicates statistical significance with $^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.001$). (C–D) Protein expression of IL-2Rγ in keratinocytes monolayers (C) and RHE (D) using primary keratinocytes exposed for 48 h to IL-4 and/or IL-13 (50 ng/mL), or not. Level of protein was assessed through Western blotting. Lymphocytes are used as positive control. RPL13a was used as loading control ($n = 2$ (C); $n = 3$ (D)). (E–G) Localization of IL-2Rγ mRNA in RHE embedded in paraffin and exposed for 48 h to IL-4 and/or IL-13 (50 ng/mL), or not (E), and in healthy or AD skin frozen in OCT (F, G). IL-2Rγ mRNA was localized through in situ hybridization and hemalun counterstaining. (G) IL-2Rγ mRNA was localized through fluorescent in situ hybridization (orange). LCA was labelled by immunofluorescence (green) and nuclei stained using RNAscope Multiplex FL V2 DAPI (323108) (blue). Insert shows control without primary antibody. Dashed lines represent limit between epidermis and dermis. Scale bars = 20 μm (E) or 50 μm (F, G). AD, atopic dermatitis; Ctrl, control; IL, interleukins; LCA, Leukocyte Common Antigen; RHE, reconstructed human epidermis.



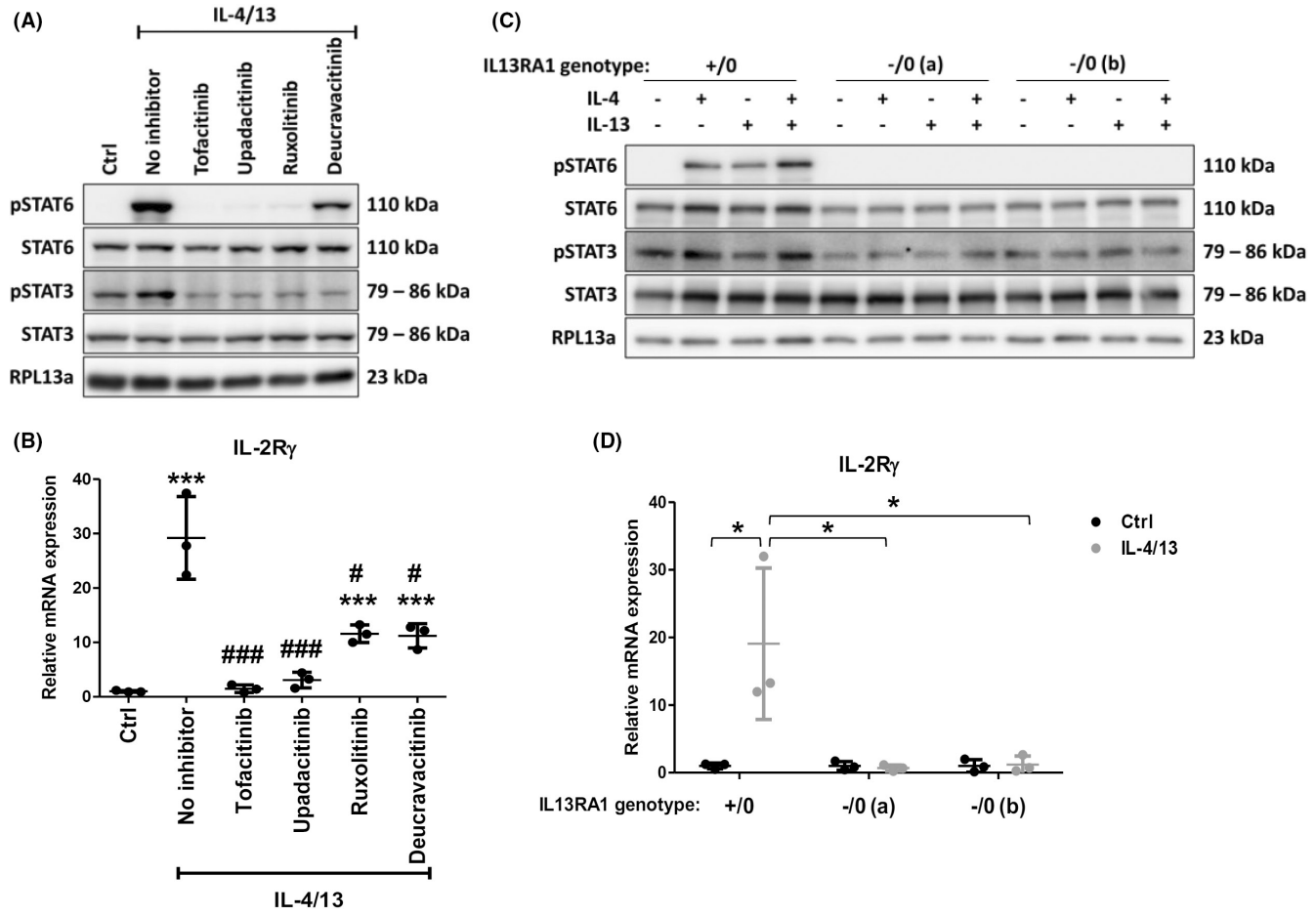


FIGURE 2 The IL-2Rγ expression triggered by IL-4 and IL-13 is suppressed by JAK inhibitors or by deletion of IL13RA1 gene. (A–C) Activation of STAT6 and STAT3 in RHE exposed to IL-4 and IL-13 (50 ng/mL) for 15 min after an overnight treatment, or not, with 1 μM tofacitinib, 0.2 μM upadacitinib, 1 μM ruxolitinib or 1 μM deucravacitinib (A) and in IL13RA1$^{+/0}$ and IL13RA1$^{-/0}$ RHE exposed for 15 min to IL-4 and/or IL-13 (50 ng/mL) (grey dots), or in control media (black dots) (C). Total and phosphorylated forms of STAT6 and STAT3 were analyzed through Western blotting. RPL13a was used as loading control. Images are representatives of three independent experiments. (B–D) Relative mRNA expression of IL-2Rγ in RHE exposed to IL-4 and IL-13 (50 ng/mL) for 48 h after an overnight treatment, or not, with 1 μM tofacitinib, 0.2 μM upadacitinib, 1 μM ruxolitinib or 1 μM deucravacitinib (B) and in IL13RA1$^{+/0}$ and IL13RA1$^{-/0}$ RHE exposed for 48 h to IL-4 and/or IL-13 (50 ng/mL) (grey dots), or in control media (black dots) (D). Level of mRNA was assessed through RT-qPCR. RPLP0 was used as reference gene. Values are expressed relative to Ctrl unexposed to IL-4 and IL-13 (B) or IL13RA1$^{+/0}$ Ctrl (*d*) (mean ± SD, $n = 3$, one-way ANOVA, * and # indicate statistical significance compared with Ctrl and No inhibitor, respectively (b) with $^{\#}p < 0.05$, $^{***/\#\#\#}p < 0.001$. * indicates statistical significance of measurement with $^{*}p < 0.05$). Ctrl, control; IL, interleukins; pSTAT, phosphorylated signal transducers and activators of transcription; RHE, reconstructed human epidermis.

to incubation with IL-4 and IL-13 of RHE made of N/TERT keratinocytes are very similar to those observed with RHE made of primary keratinocytes.[22] Indeed, RHE produced by N/TERT keratinocytes exhibit increased expression of CA2 and NELL2 and decreased expression of LOR and FLG when exposed to IL-4 and IL-13 (Figure S5B). They further exhibit enhanced expression of IL-2Rγ in RHE exposed to both interleukins, whereas this expression remains unaltered in monolayers (Figure 1H–I). To note, expression of IL-13Rα1 and IL-4Rα is never altered after exposure to IL-4 and IL-13 in RHE, produced with either primary keratinocytes or the N/TERT cell line (Figure S5C).

Immortalized N/TERT keratinocytes allow easier clonal selection of DNA-edited cells. The male genotype of N/TERT cells also facilitates inactivation of the IL13RA1 gene located on chromosome X. This was performed using CRISPR/Cas9 as we previously described.[28] Two different clones KO for IL13RA1 are identified, IL13RA1$^{-/0}$(a) and IL13RA1$^{-/0}$(b). Deletant clones are both able to reconstruct epidermis (Figure S6A). When IL13RA1$^{-/0}$ RHE are incubated for 15 min with IL-4 and IL-13, either individually or combined, no activation of STAT6 or upregulation of STAT3 phosphorylation is observed, as opposed to RHE prepared with IL13RA1$^{+/0}$ N/TERT cells (Figure 2C). After 48 h of exposure to IL-4 and IL-13, no increase in IL-2Rγ mRNA is detected in both IL13RA1$^{-/0}$ RHE, unlike in IL13RA1$^{+/0}$ RHE where upregulated IL-2Rγ expression results from such treatment (Figure 2D).

In IL13RA1$^{-/0}$ RHE, the expression of CA2 and NELL2 is no longer triggered after exposure to IL-4 and IL-13, and FLG or LOR expression is unaltered (Figures S5 and S6B). Barrier efficiency assessed by TEER measurement, whereas reduced in IL13RA1$^{+/0}$ RHE upon

exposure to IL-4 and IL-13, is no more affected in IL13RA1$^{-/0}$ RHE (Figure S6C). The efficiency of tight junctions in RHE was analyzed by testing the upward permeability to biotin. An efficient barrier is characterized by biotin migration up to keratinocytes in the granular layer, whereas an altered one allows biotin leakage into the cornified layer as described in previous studies.[31,32] In IL13RA1$^{+/0}$ RHE

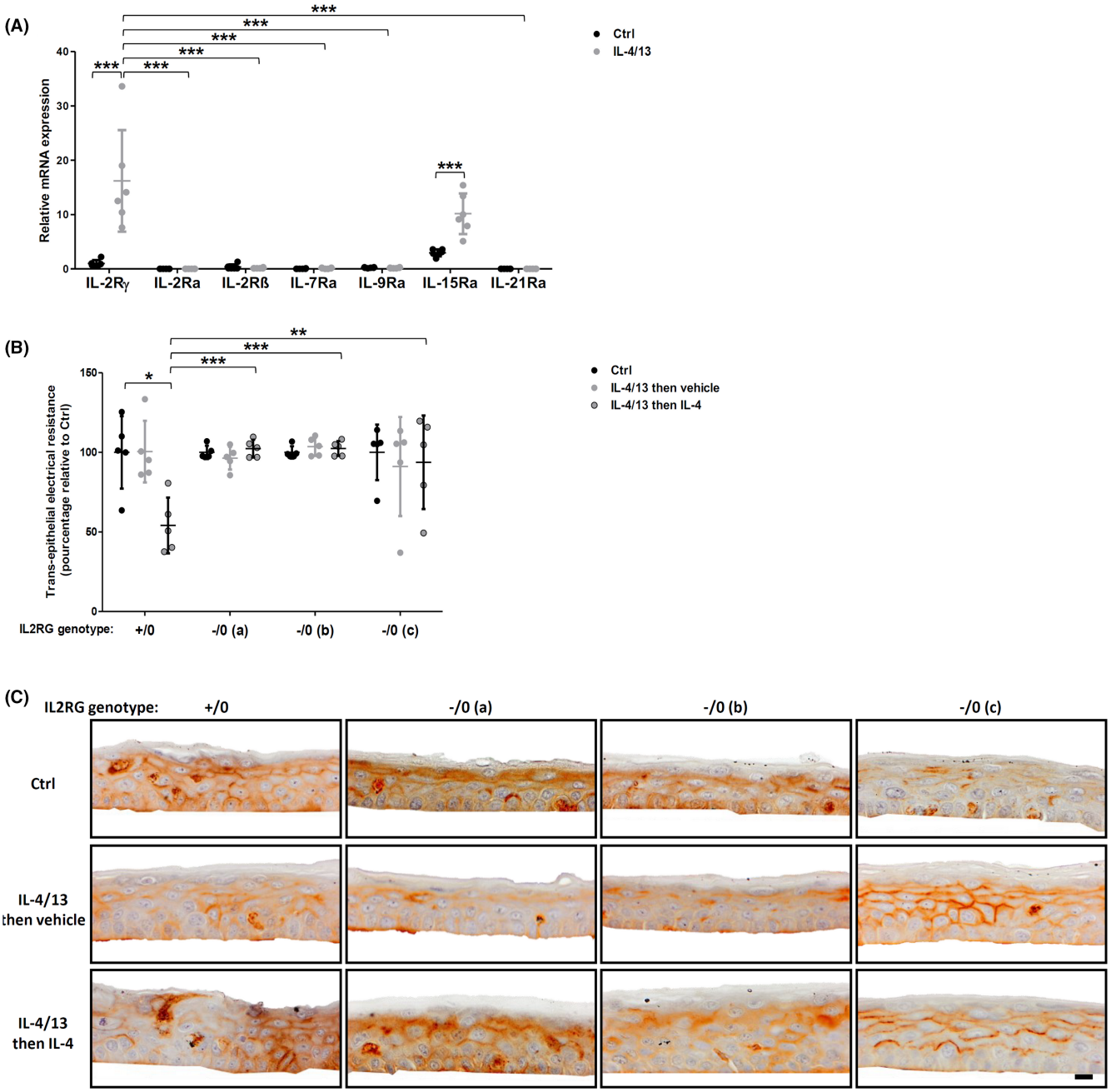


FIGURE 3 Inactivation of IL2RG gene prevents epidermal barrier alterations by IL-4 and IL-13. (A) Relative mRNA expression of IL-2Rγ, IL-2Rα, IL-2Rβ, IL-7Rα, IL-9Rα, IL-15Rα and IL-21Rα in RHE exposed for 48 h to IL-4 and IL-13 (50 ng/mL) (grey dots), or in control media (black dots). Level of mRNA was assessed through RT-qPCR. Primers with confirmed efficiency through mRNA analysis in peripheral blood mononuclear cells (PBMC). RPLP0 was used as reference gene. Values are expressed relative to Ctrl (mean ± SD, $n = 3$, one-way ANOVA, * indicates statistical significance with ***$p < 0.001$). (B, C) Barrier permeability was assessed in RHE cultured in control medium (black dots) or exposed to IL-4 and IL-13 (50 ng/mL) for 48 h and then exposed to IL-4 (50 ng/mL) (grey dots with black circle) or vehicle for 48 h (grey dots). (B) Barrier permeability was analyzed through trans-epithelial electrical resistance. Percentage values are expressed relative to Ctrl (mean ± SD, $n = 3$, one-way ANOVA, * indicates statistical significance with *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$). (C) Barrier permeability was also studied through permeability towards biotin. RHE were incubated for 30 min with biotin (2 mg/mL), fixed through acetic formol and embedded in paraffin. Biotin was revealed by streptavidin-HRP (1/200) and slides were counter-stained by hemalun. Scale bar = 10 μm. Images are representatives of two independent experiments. Ctrl, control; IL, interleukins; RHE, reconstructed human epidermis.

exposed to IL-4 and IL-13, biotin reaches the cornified layer, confirming barrier alterations. Conversely, IL13RA1$^{-/0}$ RHE do not exhibit increased permeability to biotin in those conditions (Figure S6D).

Altogether, these data indicate crucial roles for type II IL-4Rα/IL-13Rα1 receptor in keratinocytes exposed to IL-4 and IL-13, especially in terms of barrier properties and expression of AD markers, but they further reveal concomitant triggered expression of IL-2Rγ receptor subunit.

3.3 | Expression of the IL-2Rγ receptor subunit contributes to barrier alterations in RHE exposed to IL-4 and IL-13

As it happens that both IL-2Rγ and IL-4Rα can be expressed in keratinocytes of RHE, the two receptor subunits may likely dimerize to form type I receptor in the presence of IL-4. It is known however that IL-2Rγ is a common receptor subunit for IL-2, IL-4, IL-7, IL-15, and IL-21.[12] Therefore, we analyzed mRNA expression of the different subunits of these co-receptors in our model by RT-qPCR. Beside the increased expression of IL-2Rγ by IL-4 and IL-13, solely some expression of IL-15Rα is observed in reconstructed epidermis, and this expression increases when RHE are exposed for 48 h to IL-4 and IL-13 (Figure 3A). IL-15Rα binds and presents IL-15 to the dimer IL-2Rγ/IL-2Rβ to activate signalling pathways through activation of IL-2Rγ/IL-2Rβ.[33] However, although IL-15Rα is expressed in keratinocytes, this receptor subunit cannot dimerize with IL-2Rγ because IL-2Rβ and IL-15 are absent. In consequence, these data suggest that the expression of the IL-2Rγ subunit in keratinocytes solely allows association with IL-4Rα to form type I receptor for IL-4 binding.

Since IL-2Rγ subunit is expressed in keratinocytes, we again used the CRISPR/Cas9 technology and N/TERT keratinocytes to delete the corresponding gene and three different knock-out clones, IL2RG$^{-/0}$(a), IL2RG$^{-/0}$(b) and IL2RG$^{-/0}$(c), were identified. Transcripts encoding IL-2Rγ were analyzed by RT-qPCR with two primer pairs, one targeting IL-2Rγ mRNA in exon 5, and the other pair targeting in exon 1 and 2, upstream of the deletion. Both primer pairs illustrate increased expression in IL2RG$^{+/0}$ RHE after exposure to IL-4 and IL-13. Conversely, this expression remains undetected in IL2RG$^{-/0}$ RHE, proving inactivation of IL2RG gene (Figure S7A).

To initially create conditions that trigger expression of IL-2Rγ, IL2RG$^{+/0}$ RHE and IL2RG$^{-/0}$ RHE are first exposed to IL-4 and IL-13 for 48 h. They are then incubated or not with IL-4 alone, for additional 48 h to potentially activate a hypothetical type I IL-4Rα/IL-2Rγ receptor, whilst keeping IL-4Rα/IL-13Rα1 receptor activated (Figure S7B). The relative mRNA expression of CA2, and of NELL2, is upregulated in both IL2RG$^{+/0}$ and IL2RG$^{-/0}$ RHE exposed to ILs. No alteration of LOR and FLG mRNA expression is observed in any condition (Figure S7C). These data suggest that expression of IL-2Rγ in RHE has limited consequences on gene expression.

As shown by TEER measurement and biotin permeability assays, IL2RG$^{+/0}$ RHE exposed to IL-4 and IL-13, followed by additional 48 h exposure to IL-4, exhibit altered barrier properties compared to untreated RHE or RHE exposed to IL-4 and IL-13, and then to vehicle. Surprisingly, no such alteration is observed in every IL2RG$^{-/0}$ RHE despite the presence of type II receptor (Figure 3B,C). Altogether, these results suggest that IL-2Rγ subunit on keratinocytes might be involved in epidermal barrier alterations produced by IL-4 and IL-13.

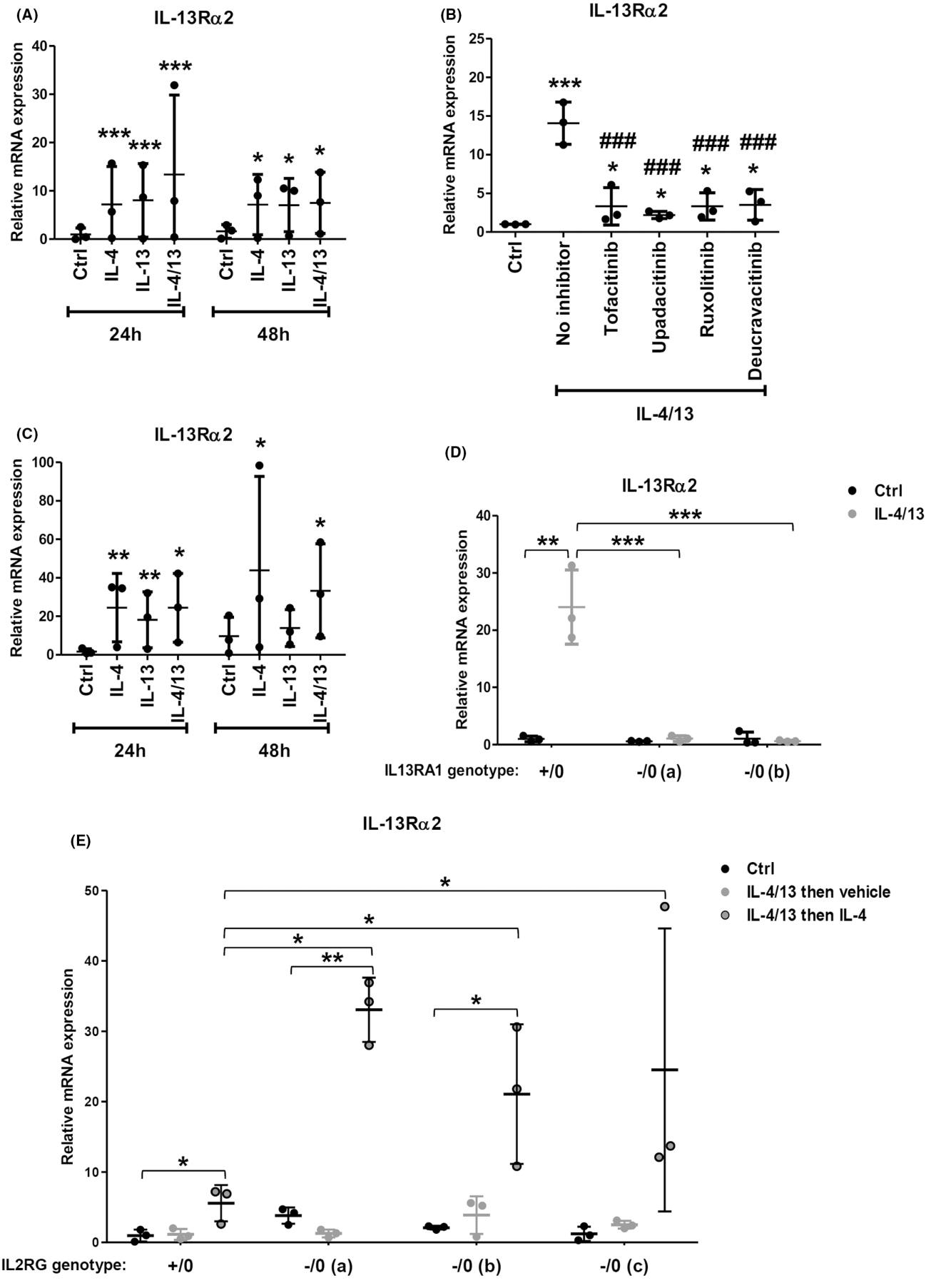
3.4 | IL-2Rγ receptor subunit reduces induction by IL-4 and IL-13 of IL-13Rα2 expression in RHE

IL-13Rα2, considered as decoy receptor, becomes overexpressed in keratinocytes exposed to IL-4 and IL-13, as previously reported.[5,7,9,30] IL-13Rα2 expression by primary keratinocytes in RHE is increased when exposed to IL-4 and/or IL-13 (Figure 4A) and this regulation is blocked by JAK inhibition (Figure 4B), indicating that induction of IL-13Rα2 expression likely results from IL-4Rα/IL-13Rα1 receptor activation. Again, these data are confirmed in RHE made of N/TERT keratinocytes (Figure 4C). Not surprisingly, mRNA expression of IL-13Rα2 does not occur in IL13RA1$^{-/0}$ RHE exposed to ILs (Figure 4D). However, when keratinocyte expression of mRNA encoding IL-13Rα2 was investigated in IL2RG$^{-/0}$ RHE first exposed to IL-4 and IL-13, then treated by IL-4 only, a strong induction is then observed, whereas only a weak expression of IL-13Rα2 is detected in IL2RG$^{+/0}$ RHE (Figure 4E). These results indicate that IL-2Rγ receptor subunit expressed in RHE might exert inhibitory control on IL-13Rα2 expression by keratinocytes, drawing attention on consequences produced by IL-13Rα2 on the epidermal barrier in AD.

4 | DISCUSSION

Among cytokines produced during Th2 immune response in skin, IL-4 and IL-13 weaken epidermal barrier properties by inducing

FIGURE 4 Induction of IL-13Rα2 expression by type II IL-4Rα/IL-13Rα1 receptor is partially inhibited by IL-2Rγ receptor subunit. Relative mRNA expression of IL-13Rα2 in RHE using primary (A) or N/TERT keratinocytes (C) exposed to IL-4 and/or IL-13 (50 ng/mL) for 24 and 48 h, in RHE using primary cells exposed to IL-4 and IL-13 (50 ng/mL) for 48 h after an overnight treatment, or not, with 1 μM tofacitinib, 0.2 μM upadacitinib, 1 μM ruxolitinib or 1 μM deucravacitinib (B), in IL13RA1$^{+/0}$ and IL13RA1$^{-/0}$ RHE exposed for 48 h to IL-4 and/or IL-13 (50 ng/mL) (grey dots), or in control media (black dots) (D) and in IL2RG$^{+/0}$ and IL2RG$^{-/0}$ RHE in control medium (black dots) or exposed to IL-4 and IL-13 (50 ng/mL) for 48 h and then were exposed to IL-4 (50 ng/mL) (grey dots with black circle) or vehicle for 48 h (grey dots). Then, mRNA levels were assessed through RT-qPCR. RPLP0 was used as reference gene. Values are expressed relative to Ctrl 24 h (A, C), Ctrl unexposed to IL-4 and IL-13 (B), IL13RA1$^{+/0}$ (D) or IL2RG$^{+/0}$ (E) Ctrl (mean ± SD, $n = 3$, one-way ANOVA, * and # indicate statistical significance compared with Ctrl (A–C) and No inhibitor (B), respectively with *$p < 0.05$, **$p < 0.005$, ***/###$p < 0.001$. (D, E) * indicates statistical significance with *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$). Ctrl, control; IL, interleukins; RHE, reconstructed human epidermis.



altered phenotype in keratinocytes. Experimentally, this alteration is reproduced in RHE exposed to both interleukins as such conditions enhance expression of CA2 and NELL2 AD markers, and lower expression of differentiation markers and barrier components. As a result, IL-4 and IL-13 induce barrier weakness, spongiosis, and hypogranulosis in RHE. Because the RHE model holds no immune cells, consequences of IL-4 and IL-13 on keratinocytes are discriminated.[20,21,33] IL-4 and IL-13 interact with RHE essentially through type II IL-4Rα/IL-13Rα1 receptor since this is the only receptor for these ligands constitutively expressed on normal keratinocytes. IL-4 and IL-13 respectively also bind to IL-4Rα/IL-2Rγ or to decoy IL-13Rα2 receptors.[10,11,34,35] Recently, induced expression of IL-2Rγ by keratinocytes in RHE exposed to IL-4 and IL-13 was interestingly reported.[23] This study first depicts conditions required for expression of IL-2Rγ by keratinocytes exposed to IL-4 and IL-13, then second investigates putative roles for IL-2Rγ receptor subunit.

Despite no expression of IL-2Rγ was detected in keratinocyte monolayers, expression of this receptor subunit was initiated inside RHE exposed to IL-4 and IL-13 in some keratinocytes among all living layers. This concurs with recent studies which have either indirectly demonstrated that IL-2Rγ functions in keratinocytes in vivo[36] or proven IL-2Rγ expression and function at the surface of cultured keratinocytes.[37] Epidermal expression of IL-2Rγ also happens in skin lesions of AD patients, in accordance with previous report.[38]

IL-2Rγ expression in keratinocytes takes place through activation of type II IL-4Rα/IL-13Rα1 receptor and downstream signalling. Indeed, JAK inhibitors interfere, although differentially, with this induction. Among them, individual characteristics might somehow explain different profiles of inhibition (tofacitinib broadly targets JAK1/2/3, upadacitinib rather targets JAK1, ruxolitinib targets JAK1/2, and deucravacitinib exhibits specificity to inhibit TYK2).[39–41] Nevertheless, they all inhibit IL-4/IL-13-induced STAT6 and STAT3 phosphorylation in RHE, hamper increased expression of AD-markers (CA2 and NELL2), or decreased expression of differentiation markers (LOR and FLG). To bring out the requirement for IL-4Rα/IL-13Rα1 receptor in keratinocytes to respond to Th2 cytokines, N/TERT immortalized keratinocytes are used to inactivate IL13RA1 gene. Like primary RHE, tissues reconstructed with N/TERT keratinocytes[25,42,43] exhibit altered phenotype when exposed to IL-4 and IL-13.[22,28] Conversely, IL13RA1$^{-/0}$ RHE created with cells deficient for IL-13Rα1 subunit fail to enhance STAT6 and STAT3 phosphorylation when exposed to IL-4 and IL-13, confirming the crucial role for type II IL-4Rα/IL-13Rα1 receptor in keratinocytes. Accordingly, no alteration of phenotype can be brought by exposure to IL-4 and IL-13 in IL13RA1$^{-/0}$ RHE. Simultaneously, the expression of IL-2Rγ receptor subunit is neither induced in IL13RA1$^{-/0}$ RHE, supporting the requirement for activated IL-4Rα/IL-13Rα1 receptor to induce IL-2Rγ expression in keratinocytes.

To analyze the aftermath of IL-2Rγ expression in keratinocytes on epidermal phenotype and barrier, IL2RG$^{-/0}$ N/TERT keratinocytes were also generated through deletions in IL2RG gene, thereby impeding any functional type I IL-4Rα/IL-2Rγ receptor that could be assembled in RHE. Indeed, we hypothesized that in presence of IL-4, IL-4Rα might dimerize with IL-2Rγ to form the type I receptor of IL-4, but more investigation is still required to prove its presence at the keratinocyte plasma membrane.

Despite some AD-like phenotype appears in IL2RG$^{-/0}$ RHE exposed to IL-4 and IL-13, excluding any major role for IL-2Rγ receptor subunit and downstream signalling in such alterations, barrier alterations were meanwhile absent following tissue exposure to IL-4 and IL-13. This unusual observation in RHE incubated with these interleukins suggests that IL-2Rγ is nevertheless somehow involved in AD-like weakened barrier. In RHE, barrier alterations may result from multiple factors, including decreased expression of FLG and LOR, alteration in lipid composition of the cornified layer, or disruption of tight junctions.[3,44–46] In our IL2RG$^{-/0}$ model, since FLG and LOR expression being unaltered by IL-4, barrier alteration does not result from abnormal differentiation.

Of interest, the other receptor for IL-13, named IL-13Rα2, is expressed in keratinocytes within AD lesional skin.[7,11] Expression of this receptor by keratinocytes in vitro is induced by IL-4 and IL-13 through binding to their type II IL-4Rα/IL-13Rα1 receptor and repressed by pretreatment of RHE with JAK inhibitors. Accordingly, IL-13Rα2 is absent from IL13RA1$^{-/0}$ RHE. To our surprise though, induction of IL-13Rα2 expression by IL-4 and IL-13 is exacerbated in IL2RG$^{-/0}$ RHE, suggesting that IL-2Rγ could somehow downregulate IL-13Rα2 expression. In the context of Th2-immune response, IL-13Rα2 competes with IL-13 for binding to IL-13Rα1, since this decoy receptor exhibits higher affinity to IL-13 than the IL-13Rα1 subunit. Because of its shorter cytoplasmic tail, IL-13Rα2 cannot activate signalling pathways like IL-13Rα1. In addition, when IL-13Rα2 subunit is present in high amount, this receptor is prone to inhibit IL-4 activation by its association to IL-4Rα.[5,9–11,47] In such context, one might hypothesize that barrier alterations by IL-4 and IL-13 is the consequence of type II IL-4Rα/IL-13Rα1 receptor activity. IL-4 and IL-13 effects are normally inhibited by IL-13Rα2. Nevertheless, IL-2Rγ in keratinocytes reduces IL-13Rα2 expression, and thereby its competition with type II IL-4Rα/IL-13Rα1 receptor (Figure S8). Further studies are now awaited to characterize roles played by IL-13Rα2 receptor in normal and pathological epidermis.

Current treatments targeting IL-4 and IL-13 in AD patients have largely illustrated the crucial importance of these cytokines and of their epidermal signalling. Indeed, anti-IL-4Rα dupilumab, and both anti-IL-13 tralokinumab and lebrikizumab have permitted an increased percentage of patients that reach 75% reduction in Eczema Area and Severity Index (EASI).[14,15,48] This study expands knowledge about IL-4 and IL-13 receptors in epidermal keratinocytes and reveals that the regulated expression of their subunit components must be considered when assessing targeted treatments. Additionally, roles potentially taken by alternative receptors for IL-4 and IL-13 might explain differences observed when treating with different biologics. Similarly, choosing particular JAK inhibitors to treat AD[17] must consider their differential effects and most specific targets. In vitro

models described herein certainly offer tools for preclinical assessment of new therapies.

In summary, this research reveals that expression of IL-2Rγ receptor subunit in keratinocytes is induced through activation of type II IL-4Rα/IL-13Rα1 receptor. IL-2Rγ expression seems required for Th2-induced alteration of the epidermal barrier. When expressed, IL-2Rγ interferes with expression of IL-13Rα2 receptor.

AUTHOR CONTRIBUTIONS

Conceptualization: AP, VDG, EDV, VGG, LD, CLDR, YP. Data curation: AP, CLDR, YP. Formal Analysis: AP. Funding acquisition: CLDR, YP. Investigation: AP, CE, AF, AFN. Methodology: AP, CE, EDV. Project administration: AP, CLDR, YP. Resource: AFN, VGG. Visualization: AP, CLDR, YP. Writing–original draft: AP, CLDR, YP. Writing–review & editing: AP, CLDR, YP.

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CONFLICT OF INTEREST STATEMENT

The authors state no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Audrey Progneaux https://orcid.org/0000-0001-9998-5257
Céline Evrard https://orcid.org/0000-0002-7450-0759
Valérie De Glas https://orcid.org/0000-0001-7366-2601
Alix Fontaine https://orcid.org/0000-0001-7412-9411
Céline Dotreppe https://orcid.org/0000-0001-9879-2517
Evelyne De Vuyst https://orcid.org/0000-0001-7152-1370
Arjen F. Nikkels https://orcid.org/0000-0001-5240-4806
Vicente García-González https://orcid.org/0000-0003-3502-2237
Laure Dumoutier https://orcid.org/0000-0002-6645-684X
Catherine Lambert de Rouvroit https://orcid.org/0000-0002-0273-8995
Yves Poumay https://orcid.org/0000-0001-5200-3367

REFERENCES

1. Cork MJ, Danby SG, Vasilopoulos Y, et al. Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol*. 2009;129(8):1892-1908.
2. De Vuyst E, Salmon M, Evrard C, Lambert de Rouvroit C, Poumay Y. Atopic dermatitis studies through In vitro models. *Front Med*. 2017;4:119.
3. Elias PM, Hatano Y, Williams ML. Basis for the barrier abnormality in atopic dermatitis: outside-inside-outside pathogenic mechanisms. *J Allergy Clin Immunol*. 2008;121(6):1337-1343.
4. Teraki Y, Sakurai A, Izaki S. IL-13/IL-22-coproducing T cells, a novel subset, are increased in atopic dermatitis. *J Allergy Clin Immunol*. 2013;132(4):971-974.
5. Shankar A, McAlees JW, Lewkowich IP. Modulation of IL-4/IL-13 cytokine signaling in the context of allergic disease. *J Allergy Clin Immunol*. 2022;150(2):266-276.
6. Omori-Miyake M, Yamashita M, Tsunemi Y, Kawashima M, Yagi J. In vitro assessment of IL-4- or IL-13-mediated changes in the structural components of keratinocytes in mice and humans. *J Invest Dermatol*. 2014;134(5):1342-1350.
7. David M, Ford D, Bertoglio J, Maizel AL, Pierre J. Induction of the IL-13 receptor alpha2-chain by IL-4 and IL-13 in human keratinocytes: involvement of STAT6, ERK and p38 MAPK pathways. *Oncogene*. 2001;20(46):6660-6668.
8. Zhang Y, He S, Mei R, et al. miR29a suppresses IL13induced cell invasion by inhibiting YY1 in the AKT pathway in lung adenocarcinoma A549 cells. *Oncol Rep*. 2018;39(6):2613-2623.
9. Andrews AL, Holloway JW, Puddicombe SM, Holgate ST, Davies DE. Kinetic analysis of the interleukin-13 receptor complex. *J Biol Chem*. 2002;277(48):46073-46078.
10. Andrews AL, Nasir T, Bucchieri F, Holloway JW, Holgate ST, Davies DE. IL-13 receptor alpha 2: a regulator of IL-13 and IL-4 signal transduction in primary human fibroblasts. *J Allergy Clin Immunol*. 2006;118(4):858-865.
11. Ulzii D, Kido-Nakahara M, Nakahara T, et al. Scratching counteracts IL-13 signaling by upregulating the decoy receptor IL-13Ralpha2 in keratinocytes. *Int J Mol Sci*. 2019;20(13):3324.
12. Waickman AT, Park JY, Park JH. The common gamma-chain cytokine receptor: tricks-and-treats for T cells. *Cell Mol Life Sci*. 2016;73(2):253-269.
13. Bakker DS, van der Wal MM, Heeb LEM, et al. Early and long-term effects of Dupilumab treatment on circulating T-cell functions in patients with moderate-to-severe atopic dermatitis. *J Invest Dermatol*. 2021;141(8):1943-1953.e13.
14. Beck LA, Thaci D, Deleuran M, et al. Dupilumab provides favorable safety and sustained efficacy for up to 3 years in an open-label study of adults with moderate-to-severe atopic dermatitis. *Am J Clin Dermatol*. 2020;21(4):567-577.
15. Wollenberg A, Blauvelt A, Guttman-Yassky E, et al. Tralokinumab for moderate-to-severe atopic dermatitis: results from two 52-week, randomized, double-blind, multicentre, placebo-controlled phase III trials (ECZTRA 1 and ECZTRA 2). *Br J Dermatol*. 2021;184(3):437-449.
16. Goncalves F, Freitas E, Torres T. Selective IL-13 inhibitors for the treatment of atopic dermatitis. *Drugs Context*. 2021;10:2021-1-7.
17. Chovatiya R, Paller AS. JAK inhibitors in the treatment of atopic dermatitis. *J Allergy Clin Immunol*. 2021;148(4):927-940.
18. Sanchez-Borges M, Capriles-Hulett A, Ortega-Martell JA, Zubeldia IA. New and potential treatments for atopic dermatitis: biologicals and small molecules. *Curr Allergy Asthma Rep*. 2019;19(3):18.
19. Frankart A, Malaisse J, De Vuyst E, Minner F, de Rouvroit CL, Poumay Y. Epidermal morphogenesis during progressive in vitro 3D reconstruction at the air-liquid interface. *Exp Dermatol*. 2012;21(11):871-875.
20. De Vuyst E, Giltaire S, Lambert de Rouvroit C, et al. Methyl-beta-cyclodextrin concurs with interleukin (IL)-4, IL-13 and IL-25 to induce alterations reminiscent of atopic dermatitis in reconstructed human epidermis. *Exp Dermatol*. 2018;27(4):435-437.
21. do Nascimento Pedrosa T, De Vuyst E, Mound A, Lambert de Rouvroit C, Maria-Engler SS, Poumay Y. Methyl-beta-cyclodextrin treatment combined to incubation with interleukin-4 reproduces major features of atopic dermatitis in a 3D-culture model. *Arch Dermatol Res*. 2017;309(1):63-69.
22. Smits JPH, Niehues H, Rikken G, et al. Immortalized N/TERT keratinocytes as an alternative cell source in 3D human epidermal models. *Sci Rep*. 2017;7(1):11838.

23. Park AY, Bourtembourg M, Chretien A, et al. Modulation of gene expression in a sterile atopic dermatitis model and inhibition of Staphylococcus aureus adhesion by fucoidan. *Dermatopathology*. 2021;8(2):69-83.
24. Minner F, Herphelin F, Poumay Y. Study of epidermal differentiation in human keratinocytes cultured in autocrine conditions. *Methods Mol Biol*. 2010;585:71-82.
25. Dickson MA, Hahn WC, Ino Y, et al. Human keratinocytes that express hTERT and also bypass a p16(INK4a)-enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics. *Mol Cell Biol*. 2000;20(4):1436-1447.
26. Bertrand-Vallery V, Boilan E, Ninane N, et al. Repeated exposures to UVB induce differentiation rather than senescence of human keratinocytes lacking p16(INK-4A). *Biogerontology*. 2010;11(2):167-181.
27. De Vuyst E, Charlier C, Giltaire S, De Glas V, de Rouvroit CL, Poumay Y. Reconstruction of normal and pathological human epidermis on polycarbonate filter. *Methods Mol Biol*. 2014;1195:191-201.
28. Evrard C, Faway E, De Vuyst E, et al. Deletion of TNFAIP6 gene in human keratinocytes demonstrates a role for TSG-6 to retain hyaluronan inside epidermis. *JID Innov*. 2021;1(4):100054.
29. Poumay Y, Pittelkow MR. Cell density and culture factors regulate keratinocyte commitment to differentiation and expression of suprabasal K1/K10 keratins. *J Invest Dermatol*. 1995;104(2):271-276.
30. LaPorte SL, Juo ZS, Vaclavikova J, et al. Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell*. 2008;132(2):259-272.
31. Yuki T, Hachiya A, Kusaka A, et al. Characterization of tight junctions and their disruption by UVB in human epidermis and cultured keratinocytes. *J Invest Dermatol*. 2011;131(3):744-752.
32. Niehues H, Schalkwijk J, van Vlijmen-Willems I, et al. Epidermal equivalents of filaggrin null keratinocytes do not show impaired skin barrier function. *J Allergy Clin Immunol*. 2017;139(6):1979-1981.e13.
33. Silverberg JI, Kantor R. The role of interleukins 4 and/or 13 in the pathophysiology and treatment of atopic dermatitis. *Dermatol Clin*. 2017;35(3):327-334.
34. Jiang H, Harris MB, Rothman P. IL-4/IL-13 signaling beyond JAK/STAT. *J Allergy Clin Immunol*. 2000;105(6 Pt 1):1063-1070.
35. Ul-Haq Z, Naz S, Mesaik MA. Interleukin-4 receptor signaling and its binding mechanism: a therapeutic insight from inhibitors tool box. *Cytokine Growth Factor Rev*. 2016;32:3-15.
36. Kamiya S, Ikegami I, Yanagi M, et al. Functional interplay between IL-9 and peptide YY contributes to chronic skin inflammation. *J Invest Dermatol*. 2022;142:3222-3231.e5.
37. Nowak K, Linzner D, Thrasher AJ, Lambert PF, Di WL, Burns SO. Absence of gamma-chain in keratinocytes alters chemokine secretion, resulting in reduced immune cell recruitment. *J Invest Dermatol*. 2017;137(10):2120-2130.
38. Plager DA, Leontovich AA, Henke SA, et al. Early cutaneous gene transcription changes in adult atopic dermatitis and potential clinical implications. *Exp Dermatol*. 2007;16(1):28-36.
39. Furumoto Y, Gadina M. The arrival of JAK inhibitors: advancing the treatment of immune and hematologic disorders. *BioDrugs*. 2013;27(5):431-438.
40. Parmentier JM, Voss J, Graff C, et al. In vitro and in vivo characterization of the JAK1 selectivity of upadacitinib (ABT-494). *BMC Rheumatol*. 2018;2:23.
41. Danese S, Peyrin-Biroulet L. Selective tyrosine kinase 2 inhibition for treatment of inflammatory bowel disease: new Hope on the rise. *Inflamm Bowel Dis*. 2021;27(12):2023-2030.
42. Moran MC, Pandya RP, Leffler KA, Yoshida T, Beck LA, Brewer MG. Characterization of human keratinocyte cell lines for barrier studies. *JID Innov*. 2021;1(2):100018.
43. Rheinwald JG, Hahn WC, Ramsey MR, et al. A two-stage, p16(INK4A)- and p53-dependent keratinocyte senescence mechanism that limits replicative potential independent of telomere status. *Mol Cell Biol*. 2002;22(14):5157-5172.
44. Berdyshev E, Goleva E, Bronova I, et al. Lipid abnormalities in atopic skin are driven by type 2 cytokines. JCI. *Insight*. 2018;3(4):e98006.
45. Kirschner N, Rosenthal R, Furuse M, Moll I, Fromm M, Brandner JM. Contribution of tight junction proteins to ion, macromolecule, and water barrier in keratinocytes. *J Invest Dermatol*. 2013;133(5):1161-1169.
46. Yokouchi M, Kubo A, Kawasaki H, et al. Epidermal tight junction barrier function is altered by skin inflammation, but not by filaggrin-deficient stratum corneum. *J Dermatol Sci*. 2015;77(1):28-36.
47. Tabata Y, Khurana Hershey GK. IL-13 receptor isoforms: breaking through the complexity. *Curr Allergy Asthma Rep*. 2007;7(5):338-345.
48. Simpson EL, Flohr C, Eichenfield LF, et al. Efficacy and safety of lebrikizumab (an anti-IL-13 monoclonal antibody) in adults with moderate-to-severe atopic dermatitis inadequately controlled by topical corticosteroids: a randomized, placebo-controlled phase II trial (TREBLE). *J Am Acad Dermatol*. 2018;78(5):863-871.e11.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1 Deletions of IL13RA1 gene using Crispr/Cas9 method in N/TERT keratinocytes.

Figure S2 Deletions of IL2RG gene using Crispr/Cas9 method in N/TERT keratinocytes.

Figure S3 Unlike RHE, differentiated keratinocytes fail to induce IL-2Rγ expression.

Figure S4 The effect of IL-4 and IL-13 on expression of AD markers is abolished by exposure of RHE to JAK inhibitors.

Figure S5 Epidermis reconstructed with immortalized N/TERT keratinocytes respond in a similar way to IL-4 and IL-13 as epidermis reconstructed with primary keratinocytes.

Figure S6 Inactivation of IL13RA1 gene prevents AD markers and epidermal barrier alterations by IL-4 and IL-13.

Figure S7 Expression of AD markers is similar in $IL2RG^{-/0}$ and $IL2RG^{+/0}$ RHE.

Figure S8: Schematic representation of inhibitory effect by IL-13Rα2 on IL-4 and IL-13 signalling.

Data S1 Supplementary Methods.

Table S1 sgRNA used for gene editing by CRISPR-Cas9.

Table S2 Primers used for amplification and sequencing of genomic DNA.

Table S3 Primers used for RT-qPCR.

Table S4 Antibodies used for western blotting.

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