

Interleukin 34 exerts anti-inflammatory activities on keratinocytes and is down-regulated in psoriatic-inflamed skin

**Magali Croquette^{1,2,3}, Alicia Faugeron^{1,4}, Clémence Fonlupt^{1,2}, Julie Godet⁵, Éric Frouin^{1,5},
Martine Garcia¹, François-Xavier Bernard^{1,4}, Sevda Cordier-Dirikoc⁴, Nathalie Pedretti⁴,
Guillaume Larid¹, Laure Favot¹, Pascal Roblot², Damien Boutin⁶, Ewa Hainaut-
Wierzbicka^{1,6}, Dominique Heymann^{7,8}, Jean-Claude Lecron^{1,9}, Franck Morel¹ and Jean-
François Jégou¹**

¹Université de Poitiers, LITEC, UR15560, Poitiers, France

²Service de Médecine Interne, CHU de Poitiers, Poitiers, France

³Unité de Médecine Vasculaire, CHU de Poitiers, Poitiers, France

⁴Qima Life Sciences, Bioalternatives, Gençay, France

⁵Service d'Anatomopathologie, CHU de Poitiers, Poitiers, France

⁶Service de Dermatologie, CHU de Poitiers, Poitiers, France

⁷Nantes Université, CNRS, UMR6286, US2B, Nantes, France

⁸ Institut de Cancérologie de l'Ouest, Saint-Herblain, France

⁹Service d'Immunologie et Inflammation, CHU de Poitiers, Poitiers, France

Correspondence: Dr. Jean-François Jégou, Université de Poitiers, LITEC, UR 15560, Pôle de Biologie Santé, 1 rue Georges Bonnet, TSA 51106, 86021, Poitiers, Cedex 9, France. Email: jean-francois.jegou@univ-poitiers.fr

Abbreviations: NHDF, normal human dermal fibroblasts; NHEK, normal human epidermal keratinocytes; OSM, oncostatin M; RHE, reconstituted human epidermis; RHS, reconstituted human skin; RT-qPCR, quantitative reverse transcription-PCR.

ORCIDs

Magali Croquette : 0000-0001-7870-9915

Alicia Faugeroux : 0000-0001-7109-6263

Clémence Fonlupt : 0000-0003-4555-2071

Julie Godet: 0000-0001-9669-805X

Eric Frouin: 0000-0002-8432-3194

Martine Garcia : 0000-0003-0348-8411

François-Xavier Bernard : 0000-0002-8463-3977

Sevda Cordier-Dirikoc : 0000-0001-5107-0410

Nathalie Pedretti : 0000-0001-7496-8987

Guillaume Larid: 0000-0003-2317-2852

Laure Favot-Laforge: 0000-0001-7642-3550

Pascal Roblot: 0000-0002-3740-3503

Damien Boutin: 0000-0003-1935-2033

Ewa-Hainaut-Wierzbicka: 0000-0002-6475-4018

Dominique Heymann: 0000-0001-7777-0669

Jean-Claude Lecron: 0000-0003-3990-5213

Franck Morel: 0000-0003-3812-9304

TO THE EDITOR

Interleukin-34 (IL-34) is expressed by keratinocytes in the skin and plays a role in Langerhans cell (LC) homeostasis through its binding to the colony-stimulating factor 1 receptor (CSF-1R) (Greter et al. 2012; Wang et al. 2012). In recent years, a growing body of literature has reported its overexpression during inflammation in tissues and/or in the serum of patients with rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel diseases or Sjogren's syndrome, in association with disease severity (Munoz-Garcia et al. 2021). By contrast, some immunoregulatory properties of IL-34 have also been described in other physiopathological contexts, either by controlling the expression of proinflammatory cytokines or by promoting M2 macrophage differentiation and Treg cell functions (Guillonneau et al. 2017). Although IL-34 is mainly produced in the skin, very few studies have investigated its expression, regulation and role during skin inflammation and it is still unclear whether IL-34 has a beneficial or a deleterious role. Li *et al.* reported low serum concentrations of IL-34 in psoriasis patients similar to those of healthy controls (Li et al. 2017), without investigating IL-34 tissue expression. By contrast, Esaki *et al.* reported a reduced IL-34 expression in skin lesions of atopic dermatitis (AD) patients, suggesting that IL-34 could have immunoregulatory properties in the skin (Esaki et al., 2015).

To test this hypothesis, we have analyzed the expression of IL-34 in the skin of psoriasis patients. The clinical characteristics of the patient cohort are presented in Supplementary Table S1. The use of human skin samples for research studies was approved by the Comité de Protection des Personnes Sud Est V and the Ethical Committee of Poitiers Hospital, and was conducted according to the Declaration of Helsinki principles. Written, informed consent was collected for each psoriasis patient and healthy skin donor. Like in AD skin lesions (Esaki et al. 2015), a significant decrease of IL-34 mRNA was observed in skin lesions of psoriasis patients compared to healthy

skins (Figure 1a). After a successful biotherapy with the anti-TNF α mAb adalimumab, IL-34 expression level was partially restored in the treated cutaneous lesions. The expressions of M-CSF, the twin cytokine of IL-34, and its receptors CSF-1R, PTPRZ1 and Syndecan-1 followed the same trend (Supplementary Text and Supplementary Figure S1a). The downregulation of IL-34 in psoriatic skin was further confirmed by immunohistochemistry with a strong reduction of IL-34 staining, particularly in highly hyperplastic regions of psoriatic lesions (Figure 1b and c), suggesting that IL-34 decrease is particularly associated with the alteration of epidermal differentiation. To support this hypothesis, we report significant correlations between the cutaneous expression of IL-34 and those of genes involved in epidermal differentiation such as cytokeratin 6, 10, 16 and filaggrin (Figure 1d and Supplementary Figure S2). Although correlations between IL-34 and skin inflammation markers were observed (Supplementary Figure S2), we did not find correlation between IL-34 expression and the PASI of psoriasis patients, neither before treatment ($r = 0.08741$; $p = 0.489$) nor after treatment with adalimumab ($r = 0.114$; $p = 0.390$). As IL-34 has been described to play a unique role in the maintenance of LCs in the epidermis (Wang et al., 2012), we showed by immunohistochemistry that the number of CD207⁺ LCs was drastically reduced in the lesional skins of psoriatic patients by comparison with healthy skins (Figure 1e and f). Both CD207 mRNA and protein expressions were positively correlated to IL-34 expression (Figure 1g and h). Our findings are in agreement with the previous observation of IL-34 decrease in AD skin lesions associated with the loss of LCs (Esaki et al. 2015).

To further investigate IL-34 downregulation during psoriasis inflammation, we carried out *in vitro* studies using two-dimensional (2D) and 3D cultures of skin cells, stimulated or not by proinflammatory cytokines. In unstimulated condition, IL-34 mRNA expression was higher in reconstituted human epidermis (RHE), wherein keratinocytes are present at all stages of epidermal

differentiation, than in 2D cultures of normal human epidermal keratinocytes (NHEK) which are mostly found in an undifferentiated state (Figures 2a). We confirmed this result at the protein level by measuring IL-34 concentrations in cell lysates of 2D and 3D cultures and by immunohistochemistry on RHE and RHS sections (Figure 2 c and d). These results confirm the link between IL-34 expression and epidermal differentiation. In contrast, dermis and normal human dermal fibroblasts (NHDF) do not express IL-34 (Figures 2a, b and d). We next stimulated NHEK and RHE with a cocktail of five cytokines, called M5, composed of IL-1 α , IL-17A, oncostatin M (OSM), TNF- α and IL-22, previously described to induce a strong psoriasis-like inflammatory phenotype *in vitro* (Guilloteau et al. 2010; Rabeony et al. 2014), or with OSM alone, a cytokine known to alter profoundly epidermal differentiation (Boniface et al. 2007; Pohin et al. 2016). A significant downregulation of IL-34 was observed in inflammatory conditions induced both by the M5 cocktail and OSM alone (Figure 2e), and was correlated with the downregulation of the epidermal differentiation markers CK10 and filaggrin (Figure 2f). The expression of M-CSF, CSF-1R, PTPRZ1 and Syndecan-1 was also investigated in these *in vitro* models of keratinocyte and fibroblast cultures (Supplementary Text and Supplementary Figure S1b to c).

Finally, to investigate a possible role of IL-34 in the regulation of skin inflammation, we stimulated NHEK with OSM which alters epidermal differentiation through STAT3 signaling pathway. As expected, keratinocyte stimulation with OSM alone induced a significant STAT3 phosphorylation, unlike IL-34 stimulation which failed to activate the same pathway (Figure 2g). However, the stimulation by IL-34 reduced by 2-fold the OSM-induced STAT3 phosphorylation (Figure 2g and h). This finding demonstrates the capacity of IL-34 to regulate OSM-induced activation of keratinocytes and was confirmed on RHE by the capacity of IL-34 to counteract the effect of the proinflammatory cytokine by restoring the expression of epidermal differentiation

genes CK10 and filaggrin whose expressions were repressed by OSM stimulation (Figure 2i). Although IL-34 was also shown to regulate TNF α expression by macrophages (Xu et al. 2015; Zwicker et al. 2015), we did not observe such regulation in OSM-stimulated keratinocytes (data not shown). Together, these findings support a regulatory role of IL-34 in skin inflammation, in agreement with previous studies (Guillonnet al. 2017).

In conclusion, we report the downregulation of IL-34 in the lesional skin of psoriasis patients, like in AD. We demonstrate that IL-34 expression by keratinocytes is tightly associated with epidermal differentiation and inhibited by proinflammatory cytokines. We also show the capacity of IL-34 to counteract the proinflammatory effect of OSM on epidermal cells, supporting the recently described immunoregulatory role of IL-34. Whether IL-34 decrease could be considered as a common feature and used as a biomarker of skin inflammation needs further investigation by exploring its expression in other skin inflammatory diseases.

DATA AVAILABILITY STATEMENT

No datasets were generated or analyzed in this study

CONFLICT OF INTEREST

The LITEC laboratory has received grant fundings from AbbVie for the IMPRA clinical studies.

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AUTHOR CONTRIBUTIONS

Conceptualization: MC, CF, DH, PR, FXB, FM, JCL, JFJ; Data Curation: MC, CF, AF, JG, EF, MG, JFJ; Formal Analysis: MC, CF, JFJ; Funding Acquisition: MC, FM, JFJ; Investigation: MC, CF, AF, JG, EF, MG, SCD, NP, DB, EHW, JFJ; Methodology: MC, CF, JG, EF, MG, SCD, NP, JFJ; Project Administration: FM, JFJ; Resources: MC, CF, AF, JG, EF, SCD, NP, DB, EHW, DH, JFJ; Supervision: PR, DH, FXB, FM, JCL, JFJ; Writing - Original Draft Preparation: MC, CF, JFJ; Writing - Review and Editing: MC, CF, AF, JG, EF, MG, FXB, SCD, NP, PR, DB, EHW, DH, JCL, FM, JFJ.

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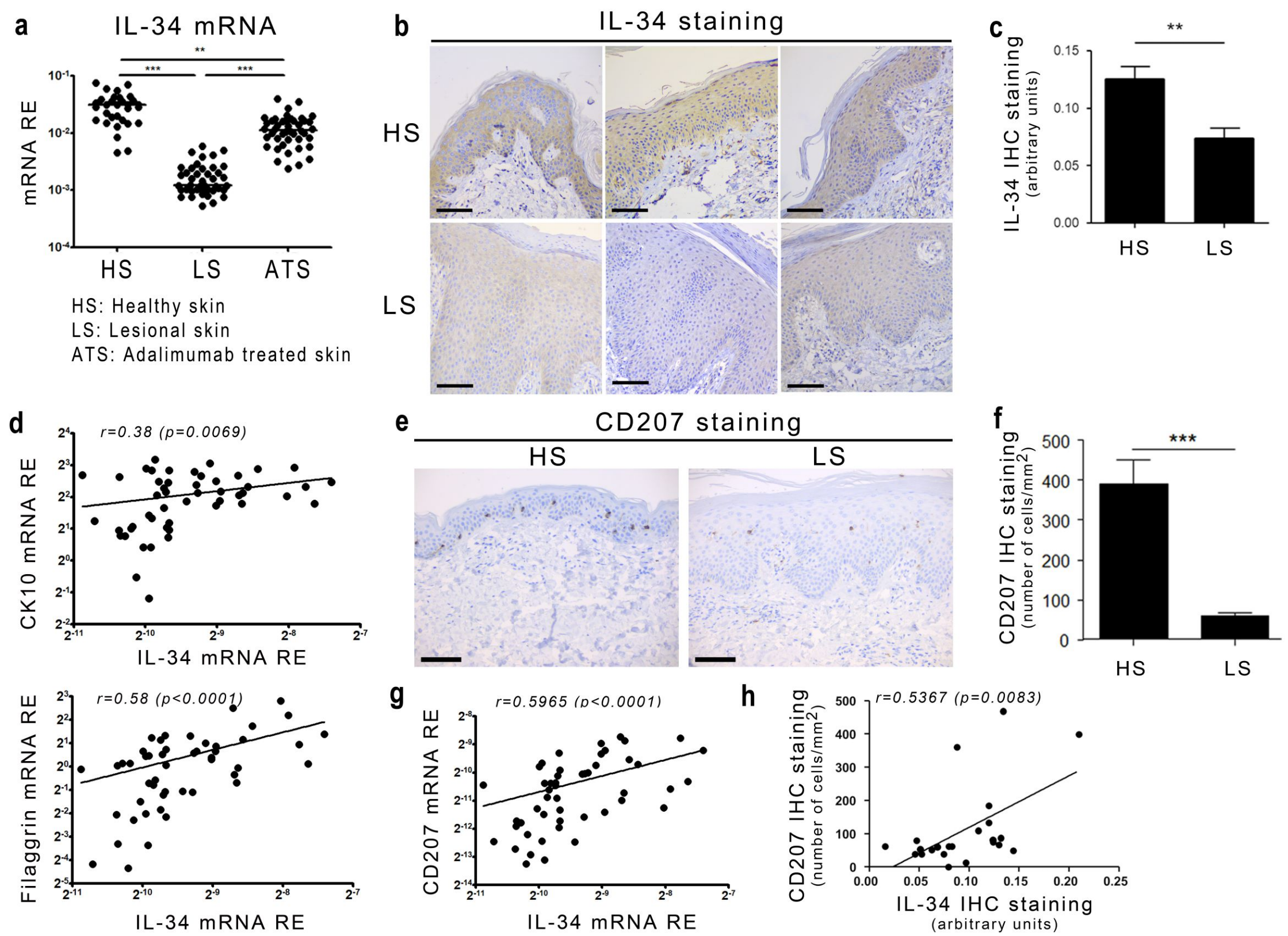
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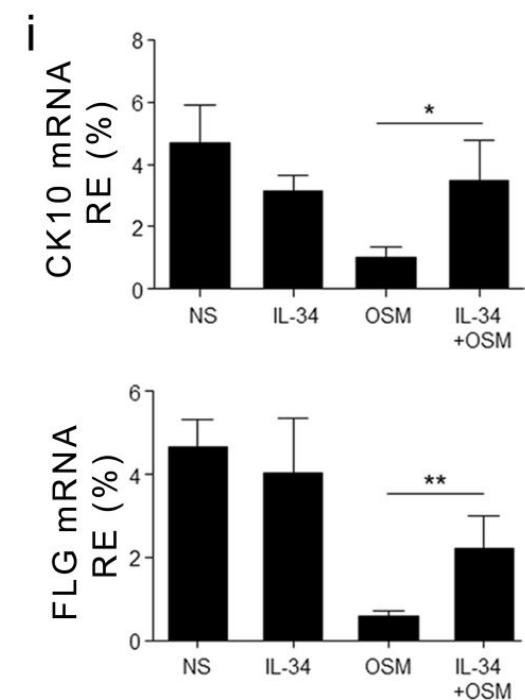
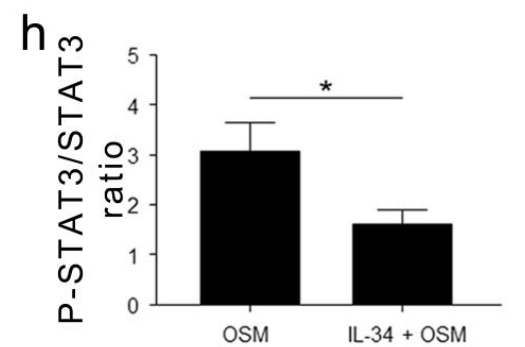
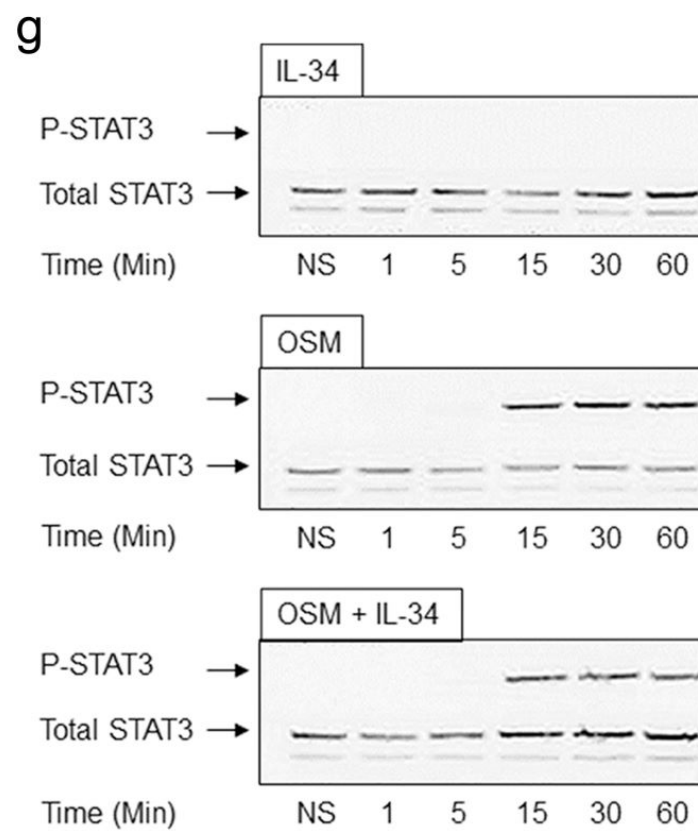
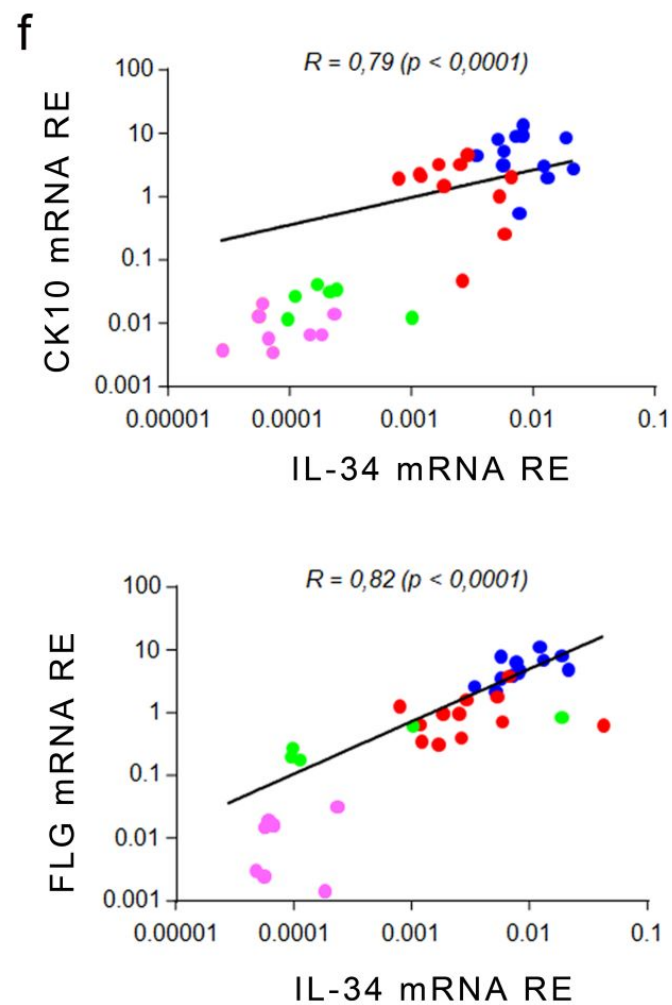
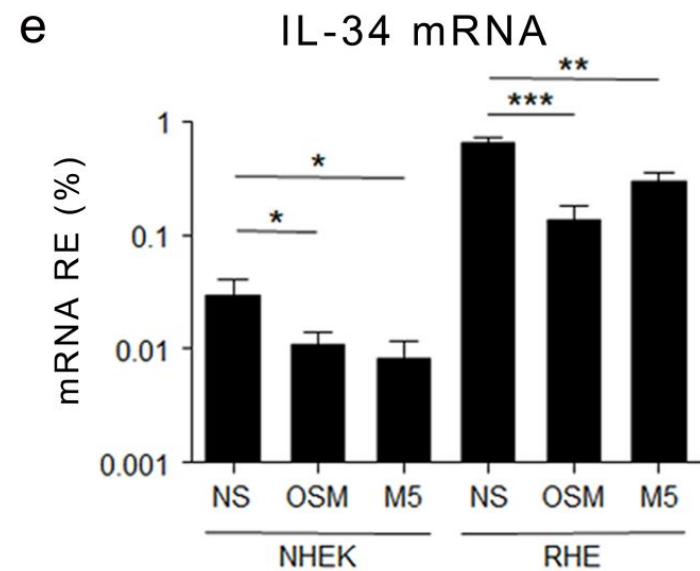
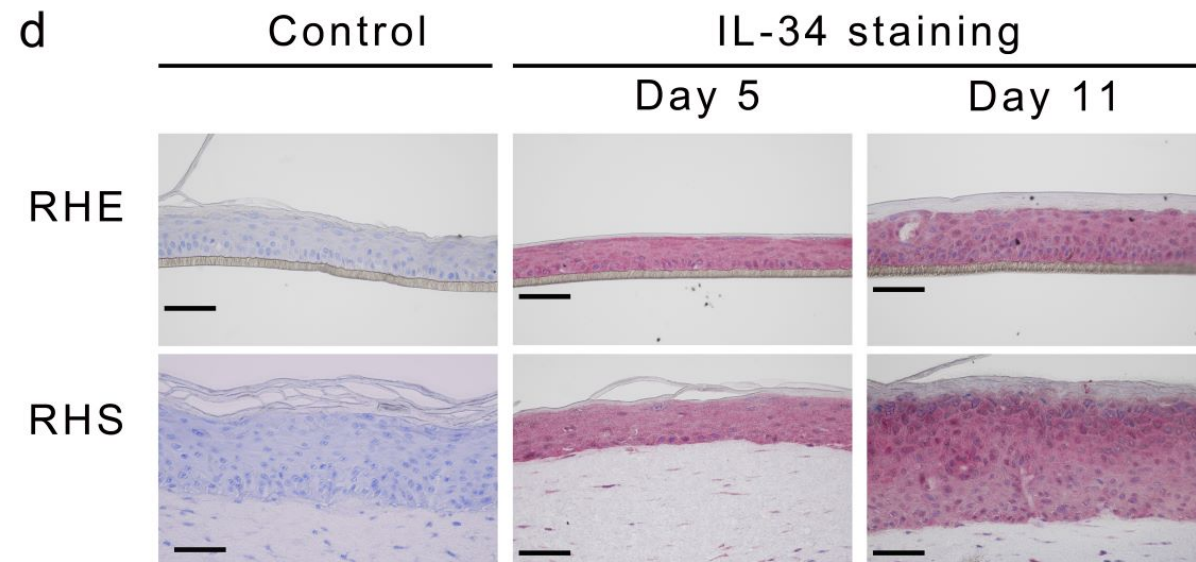
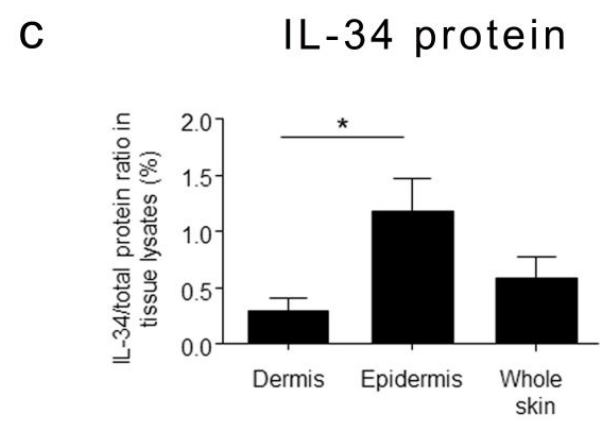
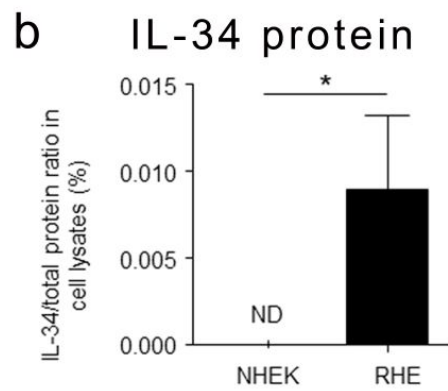
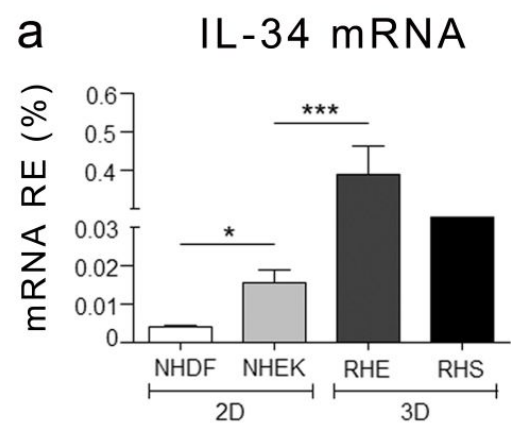
Figure 1. IL-34 expression is downregulated in psoriatic skin lesions and correlated with the loss of Langerhans cells. (a) IL-34 mRNA expression level was analyzed by RT-qPCR in skin biopsies of lesional (LS) skin of psoriasis patients (n=44) before and after four months of a successful treatment with anti-TNF α immunotherapy (adalimumab-treated skin, ATS). Healthy skins (HS) were used as controls (n=32). Data are expressed as mean \pm SEM percentage of relative expression (RE) of IL-34 to the housekeeping gene GAPDH. ** P <0.01; *** P <0.001, using one-way ANOVA with Bonferroni's multiple comparisons post-test. (b) Biopsies of healthy skins and psoriasis lesions were analyzed by immunohistochemistry (IHC) for the detection of IL-34. Representative pictures from three different patients for each group were shown; scale bar = 50 μ m. (c) Quantification of IL-34 specific staining was expressed in arbitrary units. ** P <0.01; using the Mann & Whitney's test for comparison between groups. (d) Correlations were realized between IL-34 and CK10 mRNA skin expression levels and IL-34 and filaggrin mRNA skin expression levels in psoriasis patients. R and P values are indicated and were determined using the Spearman's rank-correlation test. (e) Immunohistochemistry for the detection of the Langerhans cell marker CD207 and (f) quantification of the number of CD207-positive cells was determined per mm². *** P <0.001, using the Mann & Whitney's test for comparison between groups. Correlations between IL-34 and CD207 expressions at the mRNA level (g) and the protein level by IHC (h). R and P values are indicated and were determined using the Spearman's rank-correlation test.

Figure 2. IL-34 expression *in vitro* under healthy and proinflammatory conditions and immunoregulatory role. (a) The mRNA expression level of IL-34 was analyzed by RT-qPCR in

primary cultures of normal human dermal fibroblasts (NHDF), normal human epidermal keratinocytes (NHEK), both cultivated in monolayer (2D) and in the two 3D models of reconstituted human epidermis (RHE) and reconstituted human skin (RHS). Data are expressed as mean \pm SEM percentage of relative expression of the gene of interest to the housekeeping gene GAPDH and obtained from two to four independent experiments done in triplicate. * P <0.05; *** P <0.001, using the Mann & Whitney's test for comparison between groups. **(b)** IL-34 protein concentrations were determined by ELISA in cell lysates of NHEK or RHE and **(c)** in tissue lysates of isolated human dermis and epidermis and whole skin. The proportion of IL-34 among total proteins in the tissue lysates is expressed as mean \pm SEM percentage of IL-34 among total proteins. Data are obtained from three independent experiments. * P <0.05 using the Mann & Whitney's test for comparison between groups. **(d)** Analysis by immunohistochemistry of IL-34 staining in 3D models of RHE and RHS at day 5 and day 11 of development. Controls were realized after incubation with the secondary antibody only. Scale bar: 50 μ m. **(e)** NHEK and RHE were stimulated for 24h either with recombinant oncostatin M (OSM) at a final concentration of 10 ng/ml or the M5 cocktail of cytokines at 1 ng/ml for each cytokine, or non-stimulated (NS). IL-34 expression level was analyzed by RT-qPCR and expressed as mean \pm SEM percentage of its relative expression to the housekeeping gene GAPDH and obtained from two to four independent experiments done in triplicate * P <0.05; ** P <0.01; *** P <0.001, using the Mann & Whitney's test for comparison between groups. **(f)** Correlations were realized between IL-34 and CK10 mRNA expression levels and IL-34 and filaggrin (FLG) mRNA expression levels in unstimulated and OSM-stimulated NHEK and RHE. R and P values are indicated and were determined using the Spearman's rank-correlation test. **(g)** NHEK were stimulated with OSM (10 ng/ml), IL-34 (10 ng/ml) or both cytokines (10 ng/ml, each) for 1, 5, 15, 30 and 60 minutes before protein extraction and western blot analysis to detect phospho-STAT3 (P-STAT3) and total STAT3 signals. **(h)**

Ratios of normalized densitometry values (P-STAT3/total STAT3) were obtained from three independent western blot experiments and presented as mean \pm SEM. (i) RHE were stimulated with IL-34, OSM or both cytokines at a concentration of 10 ng/ml for 24h. The mRNA expression levels of the epidermal differentiation markers CK10 and filaggrin (FLG) were analyzed by RT-qPCR. Data are expressed as mean \pm SEM percentage of relative expression of the gene of interest to the housekeeping gene GAPDH and obtained from three independent experiments done in triplicate for each condition. * P <0.05; ** P <0.01, using the Mann & Whitney's test for comparison between groups.





● Unstimulated NHEK ● Unstimulated RHE
● OSM-stimulated NHEK ● OSM-stimulated RHE

Supplementary Materials and Methods

Skin samples from psoriasis patients

The present investigation is an ancillary study of CYTOPSO (registered in ClinicalTrials.gov as NCT01872546) and IMPRA (NCT03389984) studies in which skin inflammatory transcriptomic profiles have been studied in psoriatic patients, before and after adalimumab therapy (Buffiere-Morgado et al. 2017). These studies were conducted according to the Declaration of Helsinki principles and written informed consent was obtained from participants before inclusion. Skin biopsies were collected from lesional skin of patients with psoriasis before biotherapy and 4 months after successful treatment with adalimumab. Adalimumab was administered subcutaneously at a dose of 80 mg at week 0, and 40 mg every other week starting from week 1 to week 16. Biopsies were performed on the uninvolved (non-lesional) and lesional skin mainly localized on the back, abdomen and, at a lesser extent, arms of patients. Skin biopsies were immediately frozen in liquid nitrogen before RNA extraction or stored in formalin for histology and immunohistochemistry experiments. Healthy skin biopsies were realized from surgical samples of brachioplasty, healthy breast or abdominal skins provided by the Plastic Surgery Unit of Poitiers Hospital. The use of human skin samples for research studies was approved by the Ethical Committee of Poitiers Hospital.

Cell cultures, cytokines and reagents

Human normal epidermal keratinocytes (NHEK) and normal human dermal fibroblasts (NFDH) were obtained from surgical samples of healthy skin collected by the Plastic Surgery Unit of Poitiers Hospital. Primary cells were isolated as previously described (Boniface et al. 2005; Huguier et al. 2019). NHEK were cultured in Keratinocyte-SFM medium (Invitrogen Life Technologies, Cergy-Pontoise, France) supplemented with bovine pituitary extracts (50

µg/ml), epidermal growth factor (5 ng/ml), 100 u/ml penicillin and 100 µg/ml streptomycin. When cell cultures reached 80% of confluency, cells were starved for 18 h in SFM without supplements before stimulation. NHDF were grown in Dulbecco's-modified Eagle's medium (DMEM) supplemented with 2 mM L-glutamine, 10% fetal calf serum (FCS), 100 u/ml penicillin and 100 µg/ml streptomycin (all from Thermo Fisher Scientific, Waltham, MA, USA). After three passages, cells were starved in DMEM supplemented with 0.5 % FCS before stimulation. Reconstituted human epidermis (RHE) and reconstituted human skins (RHS) were obtained from Bioalternatives (Gençay, France).

Recombinant human IL-34 and oncostatin M (OSM) were used for cell stimulation at a final concentration of 10 ng/ml and purchased from R&D systems (Biotechne, France).

IL-34 concentrations were measured by ELISA (human IL-34 DuoSet ELISA, R&D systems, Biotechne) in culture supernatants or in cell/tissue lysates obtained after lysis in deionized water containing protease inhibitors (Protease Inhibitor Cocktail, Cell Signaling Technology) followed by a freeze/thaw cycle. For tissue lysates, an additional mechanical dissociation using a tissue grinder homogenizer followed by a sonication was performed.

RNA isolation and RT-qPCR

Total RNA was extracted from cell cultures or RHE using the Nucleospin RNA XS kit (Macherey-Nagel). RNA was reverse-transcribed with Superscript II Reverse Transcriptase (Invitrogen) and transcripts were amplified and quantified using the LightCycler-FastStart DNA Master Plus SYBR Green I kit on a LightCycler 480 instrument (Roche). Oligonucleotides were designed with Primer-Blast software, purchased from Eurogentec

(Eurogentec) and listed in Supplementary Table S2. Samples were normalized to the expression of the control housekeeping genes *GAPDH*.

Immunohistochemistry

Immunohistochemistry experiments were performed on tissue sections from formalin-fixed paraffin-embedded tissue blocks of human skin, RHE and RHS. Three-micrometer sections were cut from a tissue block, deparaffinized in xylene and hydrated in a graded series of alcohol. After antigen retrieval with citrate buffer pH6, staining was performed using monoclonal antibodies against human IL-34 (Mouse IgG2b, clone BT-34, developed and provided by D. Heymann, Nantes Université, France, 1/1000) or human CD207/Langerin (Mouse IgG2b, clone 12D6, Clinisciences, 1/100) and revealed using either the streptavidin-biotin-peroxidase method with diaminobenzidine as the chromogen (LSAB2 System-HRP, Dako) for skin sections or using the Novolink kit (Leica) with AEC as the chromogen for RHE and RHS sections. Appropriate irrelevant monoclonal antibodies were used as negative controls. Tissue sections were counterstained with hematoxylin before image acquisition using a Leica DM2000 LED microscope connected to Leica Application Suite software. For quantitative analyses, the intensity of cytoplasmic IL-34-specific staining and the number of CD207-positive cells were measured in three representative areas for each section using the Image J software (National Institute of Health, USA).

Western Blot

After stimulation with recombinant cytokines, cells were harvested at different times and lysed in RIPA buffer (50mM Tris HCL; 150 mM NaCl; 2 mM EDTA; 50 mM NaF; 1%

NP40; 0.5 % DOC; 0.1% SDS; pH 7.4). After separation on a 12% SDS-PAGE gel, proteins were transferred onto nitrocellulose membranes (Biorad) using a Trans-Blot Turbo transfer system (Biorad). Immunodetections were performed using rabbit monoclonal antibodies (all from Cell Signaling Technology) against human phospho-STAT3 (P-STAT3, clone D3A7), total STAT3 (clone 79D7) followed by incubation with anti-rabbit IgG peroxidase-conjugated polyclonal Ab (purchased from Jackson ImmunoResearch). Peroxidase activity was detected by chemiluminescence with the Immobilon Forte Western HRP substrate (Millipore) and analyzed using a LAS-3000 imaging system (Fujifilm) followed by quantification using Image J software. Images are representative of one experiment over three to four independent experiments.

Statistical analyses

Statistical analysis of significance was calculated using either the Mann & Whitney U test or the Kruskal-Wallis one-way ANOVA test with a Bonferroni post-test. The p values < 0.05 were considered as significant, and all data are represented as mean \pm SEM. Correlations studies used the Spearman test. Statistical analyses were performed using the GraphPad 5 software (Prism).

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Supplementary text

Expression of IL-34 receptors in healthy and inflammatory conditions

Interleukin-34 (IL-34) was identified as the second ligand of the colony-stimulating factor 1 receptor (CSF-1R, cFms or CD115), initially known as the receptor of the macrophage colony-stimulating factor (M-CSF or CSF-1)(Lin et al. 2008). Despite the absence of sequence homology between the two cytokines, IL-34 shares numerous functional activities with M-CSF through their binding to CSF-1R, particularly in LC development in the skin. Nevertheless, IL-34 exerts also some unique biological functions such as the maintenance of the population of LC within the epidermis (Wang and Colonna 2014). Two other receptors of IL-34 have been recently identified and thought to have regulatory activities: the receptor protein tyrosine phosphatase PTP- ζ (also known as PTPRZ1), a cell surface chondroitin sulfate proteoglycan with a constitutive phosphatase activity, which is repressed after IL-34 binding (Nandi et al. 2013) and syndecan-1 (CD138), another proteoglycan family member composed of heparan and chondroitin sulfate chains, which regulates IL-34 signaling through CSF-1R according to its cell surface expression level (Segaliny et al. 2015).

In addition to the analysis of IL-34 expression, we have extended our study to those of M-CSF, the twin cytokine of IL-34, and their common receptors in the skin of psoriasis patients. Like IL-34, the expression of M-CSF was downregulated in the lesional skin, demonstrating that IL-34 decrease in inflamed skin is not compensated by a potential increase of M-CSF (Supplementary Figure S1a). Skin expression of IL-34 receptors was also impacted during psoriasis, as demonstrated by a significant decrease in CSF-1R, PTPRZ1 and Syndecan-1 expressions in the lesional skins, suggesting that IL-34 activity in the skin might be impaired in inflammatory

conditions. Interestingly, the successful treatment of psoriasis patients with the anti-TNF α immunotherapy restored the expression of both cytokines and CSF-1R in resolved lesions, supporting the idea that IL-34 and M-CSF downregulation is reversible when the inflammatory state is reduced.

Then, we have analyzed M-CSF and IL-34 receptors expression *in vitro* in the different 2D and 3D models of skin cell cultures. A dichotomy could be observed, with dermal fibroblasts NHDF expressing mainly M-CSF and CSF-1R and keratinocytes expressing mostly IL-34 (Figure 2a) and the regulatory receptors PTPRZ1 and Syndecan-1, with a higher expression of PTPRZ1 in differentiated RHE compared to undifferentiated NHEK (Supplementary Figure S1b). Since RHE are composed of both keratinocytes and fibroblasts, they present intermediary expression profiles. Finally, we have studied *in vitro* the regulation of PTPRZ1 and Syndecan-1 expression by keratinocytes under proinflammatory conditions. A significant decrease of PTPRZ1 expression was observed in NHEK and RHE stimulated by the M5 cocktail of cytokines, while the expression of Syndecan-1 decreased only in NHEK (Supplementary Figure S1c). NHEK and RHE stimulation with OSM alone did not modify IL-34 receptor expression, excepted for RHE in which Syndecan-1 is upregulated by OSM. These findings are consistent with the reduction of IL-34 receptor expression observed in the lesional skin of psoriasis patients and confirm that the proinflammatory cytokine environment contributes to reduce keratinocyte capacity to respond to IL-34.

Together these results suggest that IL-34 have the capacity to target both cell types and is likely to have distinct biological effects in homeostatic or physiopathological conditions. Although some studies have reported the proinflammatory or profibrotic activity of IL-34 on lung fibroblasts (Zhou et al. 2018), gut fibroblasts (Franze et al. 2020) and fibroblast-like synoviocytes (Li et al. 2021), the potential biological activities of IL-34 on dermal fibroblasts remain to be explored.

Supplementary references

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Supplementary Materials and Methods

Skin samples from psoriasis patients

The present investigation is an ancillary study of CYTOPSO (registered in ClinicalTrials.gov as NCT01872546) and IMPRA (NCT03389984) studies in which skin inflammatory transcriptomic profiles have been studied in psoriatic patients, before and after adalimumab therapy (Buffiere-Morgado et al. 2017). These studies were conducted according to the Declaration of Helsinki principles and written informed consent was obtained from participants before inclusion. Skin biopsies were collected from lesional skin of patients with psoriasis before biotherapy and 4 months after successful treatment with adalimumab. Adalimumab was administered subcutaneously at a dose of 80 mg at week 0, and 40 mg every other week starting from week 1 to week 16. Biopsies were performed on the uninvolved (non-lesional) and lesional skin mainly localized on the back, abdomen and, at a lesser extent, arms of patients. Skin biopsies were immediately frozen in liquid nitrogen before RNA extraction or stored in formalin for histology and immunohistochemistry experiments. Healthy skin biopsies were realized from surgical samples of brachioplasty, healthy breast or abdominal skins provided by the Plastic Surgery Unit of Poitiers Hospital. The use of human skin samples for research studies was approved by the Ethical Committee of Poitiers Hospital.

Cell cultures, cytokines and reagents

Human normal epidermal keratinocytes (NHEK) and normal human dermal fibroblasts (NFDH) were obtained from surgical samples of healthy skin collected by the Plastic Surgery Unit of Poitiers Hospital. Primary cells were isolated as previously described (Boniface et al. 2005;

Huguier et al. 2019). NHEK were cultured in Keratinocyte-SFM medium (Invitrogen Life Technologies, Cergy-Pontoise, France) supplemented with bovine pituitary extracts (50 µg/ml), epidermal growth factor (5 ng/ml), 100 u/ml penicillin and 100 µg/ml streptomycin. When cell cultures reached 80% of confluency, cells were starved for 18 h in SFM without supplements before stimulation. NHDF were grown in Dulbecco's-modified Eagle's medium (DMEM) supplemented with 2 mM L-glutamine, 10% fetal calf serum (FCS), 100 u/ml penicillin and 100 µg/ml streptomycin (all from Thermo Fisher Scientific, Waltham, MA, USA). After three passages, cells were starved in DMEM supplemented with 0.5 % FCS before stimulation. Reconstituted human epidermis (RHE) and reconstituted human skins (RHS) were obtained from Bioalternatives (Gençay, France).

Recombinant human IL-34 and oncostatin M (OSM) were used for cell stimulation at a final concentration of 10 ng/ml and purchased from R&D systems (Biotechne, France).

IL-34 concentrations were measured by ELISA (human IL-34 DuoSet ELISA, R&D systems, Biotechne) in culture supernatants or in cell/tissue lysates obtained after lysis in deionized water containing protease inhibitors (Protease Inhibitor Cocktail, Cell Signaling Technology) followed by a freeze/thaw cycle. For tissue lysates, an additional mechanical dissociation using a tissue grinder homogenizer followed by a sonication was performed.

RNA isolation and RT-qPCR

Total RNA was extracted from cell cultures or RHE using the Nucleospin RNA XS kit (Macherey-Nagel). RNA was reverse-transcribed with Superscript II Reverse Transcriptase (Invitrogen) and transcripts were amplified and quantified using the LightCycler-FastStart DNA

Master Plus SYBR Green I kit on a LightCycler 480 instrument (Roche). Oligonucleotides were designed with Primer-Blast software, purchased from Eurogentec (Eurogentec) and listed in Supplementary Table S2. Samples were normalized to the expression of the control housekeeping genes *GAPDH*.

Immunohistochemistry

Immunohistochemistry experiments were performed on tissue sections from formalin-fixed paraffin-embedded tissue blocks of human skin, RHE and RHS. Three-micrometer sections were cut from a tissue block, deparaffinized in xylene and hydrated in a graded series of alcohol. After antigen retrieval with citrate buffer pH6, staining was performed using monoclonal antibodies against human IL-34 (Mouse IgG2b, clone BT-34, developed and provided by D. Heymann, Nantes Université, France, 1/1000) or human CD207/Langerin (Mouse IgG2b, clone 12D6, Clinisciences, 1/100) and revealed using either the streptavidin-biotin-peroxidase method with diaminobenzidine as the chromogen (LSAB2 System-HRP, Dako) for skin sections or using the Novolink kit (Leica) with AEC as the chromogen for RHE and RHS sections. Appropriate irrelevant monoclonal antibodies were used as negative controls. Tissue sections were counterstained with hematoxylin before image acquisition using a Leica DM2000 LED microscope connected to Leica Application Suite software. For quantitative analyses, the intensity of cytoplasmic IL-34-specific staining and the number of CD207-positive cells were measured in three representative areas for each section using the Image J software (National Institute of Health, USA).

Western Blot

After stimulation with recombinant cytokines, cells were harvested at different times and lysed in RIPA buffer (50mM Tris HCL; 150 mM NaCl; 2 mM EDTA; 50 mM NaF; 1% NP40; 0.5 % DOC; 0.1% SDS; pH 7.4). After separation on a 12% SDS-PAGE gel, proteins were transferred onto nitrocellulose membranes (Biorad) using a Trans-Blot Turbo transfer system (Biorad). Immunodetections were performed using rabbit monoclonal antibodies (all from Cell Signaling Technology) against human phospho-STAT3 (P-STAT3, clone D3A7), total STAT3 (clone 79D7) followed by incubation with anti-rabbit IgG peroxidase-conjugated polyclonal Ab (purchased from Jackson ImmunoResearch). Peroxidase activity was detected by chemiluminescence with the Immobilon Forte Western HRP substrate (Millipore) and analyzed using a LAS-3000 imaging system (Fujifilm) followed by quantification using Image J software. Images are representative of one experiment over three to four independent experiments.

Statistical analyses

Statistical analysis of significance was calculated using either the Mann & Whitney U test or the Kruskal-Wallis one-way ANOVA test with a Bonferroni post-test. The p values < 0.05 were considered as significant, and all data are represented as mean \pm SEM. Correlations studies used the Spearman test. Statistical analyses were performed using the GraphPad 5 software (Prism).

Supplementary references

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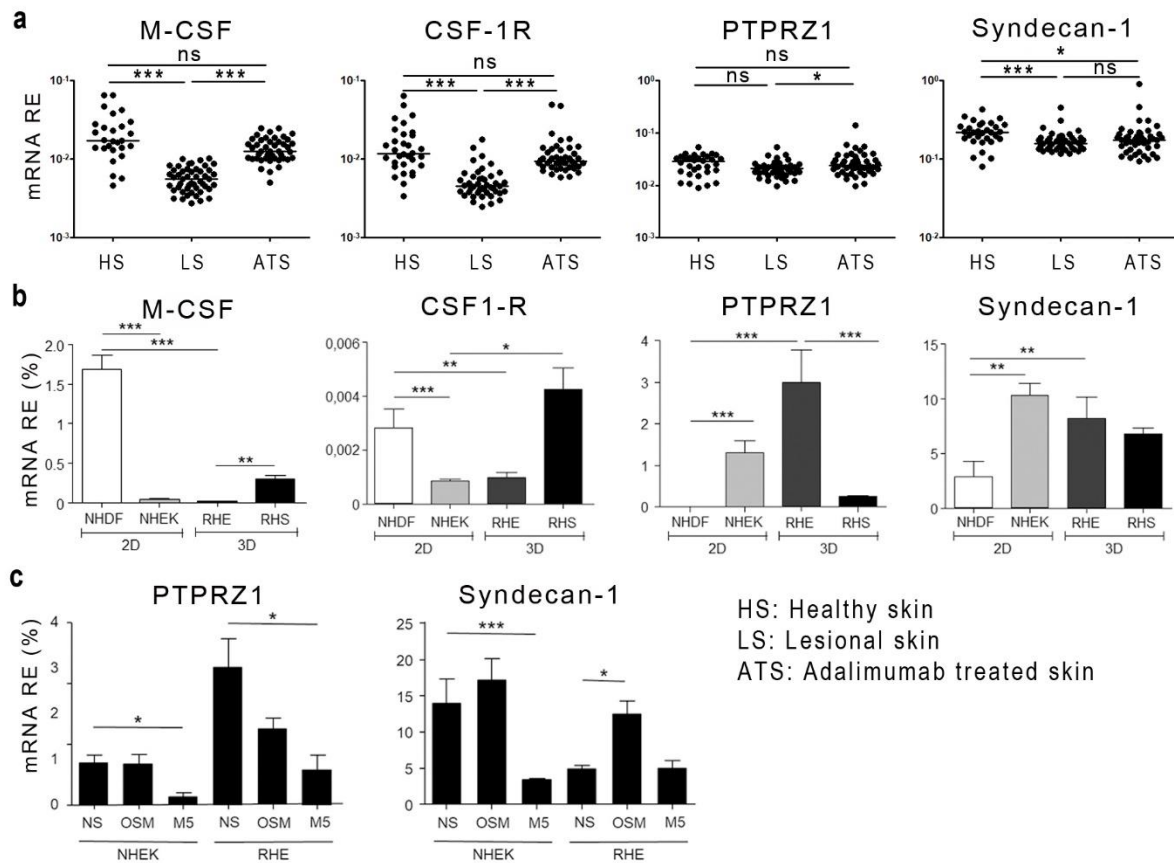
Supplementary Figure 1. Expression of M-CSF and IL-34 receptors in psoriatic skin lesions and in 2D and 3D *in vitro* models of keratinocyte cultures under healthy and inflammatory conditions. (a) The mRNA expression levels of M-CSF and IL-34 receptors including CSF-1R, PTPRZ1 and Syndecan-1 were analyzed by RT-qPCR in lesional (LS) skins of psoriasis patients (n=44) before (LS) and after four months of successful treatment with anti-TNF α immunotherapy (adalimumab-treated skin, ATS). Healthy skins (HS) were used as controls (n=32). Data are expressed as mean \pm SEM percentage of relative expression (RE) of the gene of interest to the housekeeping gene GAPDH. * P <0.05; ** P <0.01; *** P <0.001; ns, not significant, using the one-way ANOVA test with Bonferroni's multiple comparisons post-test; (b) The mRNA expression levels of M-CSF CSF-1R, PTPRZ1 and Syndecan-1 were analyzed by RT-qPCR in primary cultures of normal human dermal fibroblasts (NHDF), normal human epidermal keratinocytes (NHEK), both cultivated in monolayer (2D) and in the two 3D models of reconstituted human epidermis (RHE) and reconstituted human skin (RHS). Data are expressed as mean \pm SEM percentage of relative expression of the gene of interest to the housekeeping gene GAPDH and obtained from two to four independent experiments done in triplicate. * P <0.05; ** P <0.01; *** P <0.001, using the Mann & Whitney's test for comparison between groups. (c) NHEK and RHE were stimulated for 24h either with recombinant oncostatin M (OSM) at a final concentration of 10 ng/ml or the M5 cocktail of cytokines at 1 ng/ml for each cytokine, or non-stimulated (NS). The mRNA expression levels of IL-34, PTPRZ1 and Syndecan-1 were analyzed by RT-qPCR. Data are expressed as mean \pm SEM percentage of relative expression of the gene of interest to the housekeeping gene GAPDH and obtained from three independent experiments done in triplicate for each condition. * P <0.05; *** P <0.001, using the Mann & Whitney's test for comparison between groups.

Supplementary figure S2. Correlation between mRNA expression levels of IL-34 and genes involved in epidermal differentiation and inflammation in the lesional skin of psoriasis patients. Data were obtained from the 44 psoriasis patients of the IMPRA cohort and expressed as the mean relative expression to the housekeeping genes. Statistical analyses were performed using the Spearman's correlation test. R and *P* values are indicated for each correlation. CK, cytokeratin; BD2, β -defensin 2.

Supplementary Table S1. Clinical characteristics of the IMPRA population of psoriasis patients.

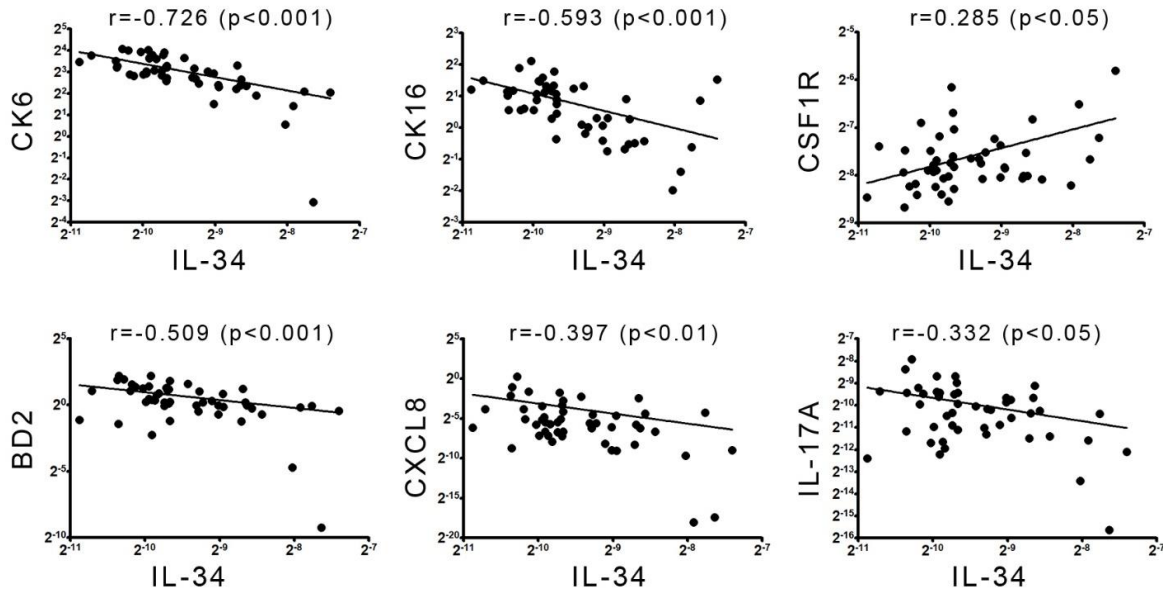
*The criteria for patient inclusion were: patient ≥ 18 years old; with plaque psoriasis with a PASI > 10 without local treatment for 2 weeks, systemic for 4 weeks, anti-TNF-alpha for 3 months or ustekinumab for 6 months; meeting indications for biotherapies (failure or contraindication of two first-line treatments: phototherapy, methotrexate or cyclosporine); without active tuberculosis detected by intradermal reaction or specific blood tests.

Supplementary Table S2. List of primer sequences used for real-time quantitative PCR.



Supplementary Figure 1. Expression of M-CSF and IL-34 receptors in psoriatic skin lesions and in 2D and 3D *in vitro* models of keratinocyte cultures under healthy and inflammatory conditions. (a) The mRNA expression levels of M-CSF and IL-34 receptors including CSF-1R, PTPRZ1 and Syndecan-1 were analyzed by RT-qPCR in lesional (LS) skins of psoriasis patients (n=44) before (LS) and after four months of successful treatment with anti-TNF α immunotherapy (adalimumab-treated skin, ATS). Healthy skins (HS) were used as controls (n=32). Data are expressed as mean \pm SEM percentage of relative expression (RE) of the gene of interest to the housekeeping gene GAPDH. *P<0.05; **P<0.01; ***P<0.001; ns, not significant, using the one-way ANOVA test with Bonferroni's multiple comparisons post-test; (b) The mRNA expression levels of M-CSF CSF-1R, PTPRZ1 and Syndecan-1 were analyzed by RT-qPCR in primary cultures of normal human dermal fibroblasts (NHDF), normal human epidermal keratinocytes (NHEK), both cultivated in monolayer (2D) and in the two 3D

models of reconstituted human epidermis (RHE) and reconstituted human skin (RHS). Data are expressed as mean \pm SEM percentage of relative expression of the gene of interest to the housekeeping gene GAPDH and obtained from two to four independent experiments done in triplicate. * P <0.05; ** P <0.01; *** P <0.001, using the Mann & Whitney's test for comparison between groups. (c) NHEK and RHE were stimulated for 24h either with recombinant oncostatin M (OSM) at a final concentration of 10 ng/ml or the M5 cocktail of cytokines at 1 ng/ml for each cytokine, or non-stimulated (NS). The mRNA expression levels of IL-34, PTPRZ1 and Syndecan-1 were analyzed by RT-qPCR. Data are expressed as mean \pm SEM percentage of relative expression of the gene of interest to the housekeeping gene GAPDH and obtained from three independent experiments done in triplicate for each condition. * P <0.05; *** P <0.001, using the Mann & Whitney's test for comparison between groups.



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Supplementary Table S1. Clinical characteristics of the IMPRA population of psoriasis patients.

	IMPRA* (n=65)
Men, n (%)	40 (62%)
Age	51 (35-60)
PASI score before adalimumab treatment	17.1 (10-50)
PASI score after 4 months of adalimumab treatment	2.7 (0-13.8)
DLQI score before adalimumab treatment	14 (5-28)
DLQI score after 4 months of adalimumab treatment	2.2 (0-16)

*The criteria for patient inclusion were: patient ≥ 18 years old; with plaque psoriasis with a PASI > 10 without local treatment for 2 weeks, systemic for 4 weeks, anti-TNF-alpha for 3 months or ustekinumab for 6 months; meeting indications for biotherapies (failure or contraindication of two first-line treatments: phototherapy, methotrexate or cyclosporine); without active tuberculosis detected by intradermal reaction or specific blood tests.

Supplementary Table S2. List of primer sequences used for real-time quantitative PCR.

Human Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
<i>IL-34</i>	AATCCGTGTTGTCCCTCTTG	CAGCAGGAGCAGTACAGCAG
<i>M-CSF</i>	GTTTGTAGACCAGGAACAGTTGAA	CGCATGGTGTCTCCATTAT
<i>CSF-1R</i>	CAACATGCCGGCAACTACT	CGCATGGTGTCTCCATTAT
<i>PTPRZ1</i>	AGACCGTCTGGAAATGCGAA	TCCATTAGCCCAATCCAGGC
<i>Syndecan-1</i>	AGCACCTGGCATCGCACCATTCTG	CCCACGAAACAAAGTGACTCCTGTC
<i>CK10</i>	GCCCCGACGGTAGAGTTCTTT	CAGAAACCACAAAACACCTTG
<i>CK16</i>	CAGCGAACTGGTACAGAGCA	CTCCACACTGCCAATCAGTC
<i>Filaggrin</i>	AGGAACAGGCAAGGTCAAGTCCAG	CACGTGTGAACTCTTGGTGGCTCT
<i>CD207</i>	AGGTCGTGTGGACAACATCA	AAAGCACTGGCTTTCTCAA
<i>GAPDH</i>	GGCTCTCCAGAACATCATCCCTGC	GGGTGTCGCTGTTGAAGTCAGAGG
<i>Actin B</i>	GGACTTCGAGCAAGAGATGG	AGGAAGGAAGGCTGGAAGAG
<i>RPL13</i>	TAAACAGGTACTGCTGGGCCGGAAGGTG	CACGTTCTTCTCGGCCTGTTCCGTAGC