

IL-19 may aggravate atopic dermatitis by enhancing the role of IL-4/IL-13 in downregulating keratinocyte barrier proteins and upregulating the secretion of proinflammatory cytokines

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Abstract

Atopic dermatitis (AD) is a relapsing inflammatory skin disorder, with characteristic T helper 2 (Th2)-based immune dysregulation and epidermal barrier function defect. In AD, keratinocyte plays a critical role in maintaining skin integrity and immune homeostasis. Interleukin (IL)-19 is a member of the IL-10 cytokine family. It can be secreted by and act on keratinocytes. Although it has been demonstrated that increased IL-19 in AD patients' lesions and serum is positively correlated with the severity of AD, the role of IL-19 in the regulation of the epidermal barrier and immune homeostasis of keratinocytes remains unclear. Thus, we aim to investigate the effect of IL-19 on the production of epidermal barrier related proteins and proinflammatory cytokines in keratinocytes, and the synergistic effect of IL-19 with IL-4/IL-13 *in vitro* experiment. In this study, barrier related proteins (filaggrin/FLG, loricrin/LOR, keratin-10), epithelium-derived cytokines (thymic stromal lymphopoietin/TSLP, IL-33, IL-25), IL-19 and the phosphorylation level of STAT3 and STAT6 were measured in HaCaT cells by RT-PCR and/or western blot before and after stimulated with IL-4/IL-13 with or without different concentrations of IL-19. The changes of IL-19 levels in HaCaT cells after stimulation with house dust mite (HDM) or staphylococcal enterotoxin type B (SEB) were also examined. We found that IL-19 could promote the production of TSLP in keratinocytes, but reduce the expression levels of LOR and keratin-10. Meanwhile, IL-19 significantly enhanced the effects of IL-4/IL-13 on keratinocytes, including inducing the expression of IL-19 and epithelial derived cytokines and inhibiting the expression of barrier proteins. These enhanced effects were accompanied by increased phosphorylation of STAT3 and STAT6, but no change in the expression level of IL-4/13 receptor. In addition, HDM but not SEB can induce keratinocytes to secrete IL-19. Taken together, IL-19 can enhance the effect of IL-4 / IL-13 on keratinocytes *in vitro*, and may play an important role in the pathogenesis and progression of AD.

Introduction

Atopic dermatitis (AD) is a chronic recurrent inflammatory skin disease with characteristic Th2-based immune dysregulation and epidermal barrier defect [26]. IL-4 and IL-13 are two central pathogenic cytokines in AD. IL-4 and IL-13 upregulation in epidermis is closely associated with decreased epidermal barrier proteins (FLG, LOR and keratin-10) and increased epithelium-derived cytokines (TSLP, IL-25 and IL-33) [10]. Interestingly, these epithelium-derived cytokines in turn activate Th2 cells via type 2 innate lymphocytes (ILCs)-2, Langerhans cells (LCs) and other cells, further exacerbating keratinocytes' pathological changes [5]. This process perpetuates AD's initiation and progression.

The global incidence of AD is increasing, carrying significant burden on healthcare resources [4]. At present, topical corticosteroids and calcineurin inhibitors are still the first-line treatment for acute flares and remission maintenance in AD, but side effects and recurrence are still two big challenges [28]. Dupilumab was the first successful biologic targeted on IL-4/IL-13 used in patients with AD [36]. Although some AD patients benefit from dupilumab therapy, 40–50% patients do not respond well to it [19], and some patients developed psoriasiform erythema in this process[9]. This suggested that AD is an extremely complex inflammatory disorder [29]. With the exception of IL-4/IL-13, other cytokines such as

IL-33, IL-17A, and IL-22, have been demonstrated to participate in the perpetuation of AD [8]. Therefore, new therapies based on endotype warrant further investigation.

IL-19 is a member of IL-10 cytokines family, which also include IL-10, IL-20, IL-22, IL-24, and IL-26[12]. By binding to IL-20RA/IL-20RB, a heterodimer receptor complex, IL-19 activates STAT3 signal in tissue cells, including keratinocytes [37]. It has long been proved that IL-19 plays a vital role in enhancing Th2 immune reaction in inflammatory disorders [17], and IL-19 induces the differentiation of naive T cell into Th2 cell [34]. Furthermore, Konrad et al. has validated that IL-19 was dramatically elevated in AD patients' serum, significantly correlated with EASI scores, and declined with skin recovery [24]. What's more, this reduction of IL-19 level in serum are often antecedent to clinical improvements evaluated by EASI score[23]. In previous study, we also found a significant increase in the level of IL-19 in AD lesions by single-cell sequence (unpublished data). These studies indicated that IL-19 is closely associated with AD.

Keratinocyte plays a critical role in maintaining skin integrity and immune homeostasis in AD. Although we have known that IL-19 could be secreted by and act on keratinocytes, the effect of IL-19 on keratinocytes is still elusive. And, as a pro-Th2 cytokines, what is the combined effects of IL-4/IL-13 with IL-19 on keratinocytes? Therefore, in this study, we first determined the level of barrier proteins and epithelium-derived cytokines in keratinocytes treated with IL-19 alone or IL-19 together with IL-4/IL-13. Afterwards, we further explored underlying molecular mechanism by measuring the phosphorylation of STAT3 and STAT6 in keratinocytes under the same stimulation conditions. In addition, considering external allergens are indispensable to the pathogenesis of AD, we investigated whether external stimuli, like HDM and SEB, could induce the production of IL-19 in keratinocyte. In conclusion, the purpose of this study is to explore the possible mechanism of IL-19 in the pathogenesis of AD, so as to further broaden our understanding of AD pathophysiology and provide prospects for endotype based therapy (see Fig. 1).

Materials And Methods

Cell culture and stimulation with cytokines

Human immortalised keratinocytes, HaCaT cells, were acquired from ATCC (order no. 300493) and were cultured in Dulbecco's modified Eagle's medium (Hyclone; sh30022.01), with antibiotics (100 Unit/mL of penicillin and 100 µg/mL of streptomycin) and 10% fetal bovine serum (Hyclone; Cat. Sh30042.01) added. HaCaT cells were regularly cultured in T75 cm² culture flasks and kept at 37°C in a humidified environment with 5% CO₂. Every 48 hours, the medium was changed. Cells were trypsinized, washed, and resuspended after confluency reached 80%. With a number of 2×10⁵ cells/ml in the culture medium seeded in 12-well plates (corning, #3513), 1 ml medium was added to every well. When the cells achieved confluence, the medium was totally removed and 1 ml serum-free medium was added to every well. Meanwhile, the cells were treated with recombinant human IL-19 (20, 40, 80 ng/ml; MCE 65397), IL-4 (20ng/ml; Cloud Clone, APA077Hu61) plus IL-13 (20ng/ml; Cloud Clone, APA060Hu01), or IL-4/IL-13 (20ng/ml) plus IL-19 (20, 40ng/ml), *S. aureus* Enterotoxin type B (SEB) (200 ng/ml; MCE, HY-P71808) and House Dust Mite (HDM) (100 ug/ml; GREE, XPB91D3A2.5. US), then incubation was completed at 37°C

and 5% CO₂. After indicated time, cells were lysed by TRIzol Reagent (TaKaRa 9108) for isolation of total mRNA, and RIPA buffer (Abclona RM00022) for isolation of total protein.

Cell-counting Kit (Cck) Assay

We use CCK assay to test cytotoxicity. HaCaT cells were maintained in 96-well plates (5×10^3 cells each well), treated with 40, 80 ng/mL IL-19, and cultured for 24 h at 37°C in a 5% CO₂ incubator. CCK reagents were added to per well, then the plates were incubated for 1 h. The absorbance was determined at 450 nm with a microplate spectrophotometer (Dibiotech Ltd., Seoul, Korea).

Extracting RNA and performing quantitative reverse transcription PCR.

Total RNA was extracted from HaCaT cells with TRIzol Reagent. Complementary DNA was synthesized using RT reagent Kit (Takara RR047A). mRNA levels were analysed using qPCR kit (ABclonal RK21203) on a PCR Amplifier (BIO RAD 788BR08211 Singapore). Primers for human GAPDH, IL-19, IL-25, IL-33, TSLP, FLG, LOR, keratin-10, IL-20RA and IL-20RB were as Table 1. We used Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression level as an internal control, to analyse all samples. The relative expression of every gene were determined using the $2^{-\Delta\Delta CT}$ method.

Western Blot Analysis

Total protein in treated HaCaT cells was extracted on ice by a total protein extraction kit containing protease inhibitors and phosphatase inhibitors. Protein containing mixture was centrifuged by high-speed centrifuge (15000 rcf, 15 minutes) at low temperature (4°C), followed by collection of the supernatant fluid. The concentration of extracted protein was identified by Bicinchoninic Acid method (Beyotime P0012S). Subsequently, protein samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel and transferred onto polyvinylidene difluoride membranes (Beyotime FFP32) at 4°C for 2 h. 5% non-fatty milk was formulated with Tris-buffered saline with Tween-20 (TBST) to block a specific antigen for 1 h. After washing 3–5 times with TBST, the membranes were conducted primary antibodies incubation, phosphor-STAT6 (Abcam ab188766, USA, Rabbit, 1:1000), TSLP (Abcam ab263947, USA, Rabbit, 1:1000), IL-19 (Abcam ab154187, USA, Rabbit, 1:1000), phosphor-STAT3 (ABclonal 4000000150, China, Rabbit, 1:1000), STAT3 (ABclonal 4000002603, China, Rabbit, 1:1000), STAT6 (ABclonal 4000000481, China, Rabbit, 1:1000), FLG (ABclonal 3561373004, China, Rabbit, 1:1000), GAPDH (cell signaling 2118, China, Rabbit, 1:10000) at 4°C overnight. Next day, we washed the membranes with TBST for 3–5 times and incubated them with secondary antibodies (Abclonal AS014, China, Goat Anti-Rabbit IgG, 1:10000) at room temperature for 2 h. Enhanced chemical luminescence method was used to visualize target proteins on the exposure machine.

Statistical analysis

We presented all values as the mean \pm standard error. Statistical analysis was performed with GraphPad prism 8.0. The significance of differences between groups were analysed by one-way ANOVA. A p-value < 0.05 was considered to have statistical significance. All experiments were performed at least three times.

Result

IL-19 reduced LOR and keratin-10 expression, but promoted TSLP expression in HaCaT cells.

In order to explore the effect of IL-19 alone on keratinocytes, we first examined related molecules in HaCaT cells stimulated with different concentrations of IL-19. Before measurement, we detected HaCaT cells viability after IL-19 treatment using the CCK-8 assay. The result revealed that IL-19 didn't affect HaCaT cells' proliferation and death compared to control group (Fig. 1a). Then, we measured the transcript levels of TSLP, IL-33, IL-25, FLG, LOR and keratin-10 in 40ng/ml, 80ng/ml IL-19-stimulated HaCaT cells for 24h. We found the decreased production of LOR and keratin-10, and increased TSLP expression in keratinocytes in response to IL-19 stimulation, as measured by RT-PCR (Fig. 1b-g). Since IL-19 signals through its receptors complex (IL-20RA/IL-20RB)[37], IL-20RA and IL-20RB were also assessed in HaCaT cells after treated with 40ng/ml IL-19. RT-qPCR analysis showed the elevation of IL-20RA/RB in HaCaT cells (Fig. 1h and i). These finding together demonstrated a partial effect of IL-19 on keratinocyte.

IL-19 Enhanced The Activity Of IL-4/il-13 On Keratinocytes

It has been widely accepted that IL-4/IL-13 are closely associated with AD [7]. And IL-19 has long been recognized as a pro-Th2 cytokines. To illuminate whether IL-19 could enhance the role of IL-4/IL-13 on keratinocytes, we first stimulated HaCaT cells with a very low concentrations (20ng/ml) of IL-4/IL-13, which didn't induce the decreased barrier related proteins and increased epithelium-derived cytokines in HaCaT cells. However, treatment with IL-19 significantly upregulated the mRNA expression of IL-25, IL-33 and TSLP, and downregulated the transcript level of FLG, LOR and keratin-10 (Fig. 2a-f). Accordingly, we further measured the FLG and TSLP proteins in HaCaT cells after treatment with 20ng/ml IL-4/IL-13 and 40ng/ml IL-19 alone or in combination. Western blot results revealed decreased FLG and increased TSLP in HaCaT cells stimulated with the three cytokines, compared to other groups (Fig. 2g).

Previous study has found that IL-4 could induce IL-19 production in HaCaT cells [3]. We next aimed to determine whether IL-19 transcript level could be further increased in keratinocytes stimulated by IL-4/IL-13 with IL-19. Using RT-qPCR, we found that IL-4/IL-13 were significantly capable of enhancing IL-19 gene expression in combination with IL-19 (Fig. 3), which may indicate a positive feedback loop that further exacerbates the effect of IL-4/IL-13 and IL-19 on keratinocytes.

Combined stimulation of IL-19 and IL-4/IL-13 can significantly promote the phosphorylation of STAT3 and STAT6.

It has been demonstrated that the activation of STAT3 could be induced by IL-19 in keratinocytes [25]. And increasing studies have revealed that IL-4/IL-13 activate related signaling pathways by targeting on STAT3/STAT6 [1]. Thus, we examined whether IL-19 augmented the effect of IL-4/IL-13 on keratinocytes through promoting STAT3 and STAT6 phosphorylation. We found enhanced STAT3 and STAT6 phosphorylation in keratinocytes stimulated with IL-19 plus IL-4/IL-13 at 30 min using western blot (Fig. 4a-d). These data indicate that IL-19 amplified IL-4/IL-13 induced keratinocytes' pathological changes by STAT3- and STAT6-dependent pathways.

In addition, we didn't find increased IL-13 receptor (IL-4R α /IL-13R α 1) or IL-19 receptor complex (IL-20RA/RB) under the treatment with IL-4/IL-13 and IL-19 (data not shown). Therefore, IL-19 promotes IL-4/IL-13 induced STAT-3/6 phosphorylation by acting on the signal pathway directly.

Hdm But Not Seb Can Induce The Expression Of Il-19 In HacaT Cell

House dust mite (HDM) sensitization and Staphylococcus aureus colonization have been shown to affect skin barrier function and type 2 immunity through complex mechanisms, thus participating in the pathogenesis of AD [30] [32]. We then ask whether these two most common exogenous antigens affect the production of IL-19 in keratinocytes. We first co-cultured human HaCaT cells with HDM for 12h, 24h, and measured the levels of IL-19 by RT-PCR and Western Blot. Compared with control group, IL-19 expression was significantly increased in HDM-stimulated HaCaT cells at 24h (Fig. 5a-c). In contrast, SEB didn't stimulate the expression of IL-19 under the same condition (data not shown). These data demonstrate that HDM, a key external allergen in AD, can enhance IL-19 production in keratinocytes.

Discussion

In order to elucidate the heterogeneity of AD's pathogenesis, our team performed single-cell sequence study on the chronic lesions of AD patients. In addition to the classical cytokines (IL-4/IL-13), we also found the elevated IL-19 level in the epidermis of AD patients (unpublished data). Studies by other researchers have shown the correlation between IL-19 and the severity of AD [24]. Although IL-19 is generally considered as a type 2 inflammatory cytokine, its role and mechanism in AD are still unclear [38]. IL-4 and IL-13, the classical type 2 cytokines, have been confirmed to be closely related to AD epidermal and systemic inflammation, and can aggravate epidermal barrier dysfunction [18]. Therefore, in this study, we aimed to determine whether IL-19 could enhance the effect of IL-4/IL-13 on keratinocytes in AD patients. In this study, we found that IL-19 could enhance the effects of IL-4 and IL-13 on keratinocytes *in vitro* experiments, including the downregulation of barrier proteins (FLG, LOR and keratin 10), and the induction of epithelial-derived inflammatory cytokines (IL-25, IL-33 and TSLP) (see Fig. 6). Overall, IL-19 significantly amplified type 2 inflammation in keratinocytes, potentially triggering the deterioration of AD disease.

Studies have shown that IL-19 can regulate the differentiation and maturation of naive T cells into Th2 cells [38]. Later, the Kragballe group reported that IL-19 was highly expressed in keratinocytes and

activated STAT3 in the diseased epidermis, including AD [35]. In AD lesions, IL-19 expression was positively correlated with EASI score and decreased before clinical improvement [24]. Therefore, elucidating the role of IL-19 in keratinocytes has important implications for AD. In our study, we first found that IL-19 alone decreased the expression of LOR and keratin-10 in HaCaT cells, but promoted TSLP production. At the same time, we observed enhanced expression of IL-19 receptor complex (IL-20RA/RB) in IL-19-treated HaCaT cells. These results indicated that IL-19 may act on keratinocytes by increasing IL-20RA/RB levels. However, when IL-19 was combined with IL-4/IL-13, the three cytokines significantly downregulated the expression of barrier proteins (FLG, LOR, keratin-10) and upregulated the level of epithelial-derived cytokines (IL-33, IL-25 and TSLP). More and more data have demonstrated that the reduction of barrier proteins is a direct factor for skin barrier defects, which facilitate the initiation and progression of AD [40]. Moreover, the effect of TSLP and IL-33 on enhancement of Th2-type immune response has been reported repeatedly in AD[11]. And IL-25 could inhibit FLG gene expression and protein level in human keratinocytes[6]. Interestingly, studies also showed that damaging the skin by physical factors could lead to increased TSLP in skin lesion, further promoting the progress of immune inflammation [20]. Although the role of IL-4/IL-13 in the induction of epithelium-derived cytokines and reduction of barrier proteins is well known, we only stimulated HaCaT cells with very low concentrations of IL-4/IL-13 [14], which didn't cause significant changes in HaCaT cells. However, these three cytokines had a significant effect on keratinocytes when they stimulated cells together. These results indicated that IL-19 is a critically provocative cytokine in the pathogenesis of AD, which is consistent with our single-cell sequence study. The subjects enrolled in our previous single-cell sequence study were all chronic and severe AD. These results suggest that IL-19 and IL4 / 13 may cooperate to participate in the chronic and severe AD. Therefore, simultaneous anti-il-19 and anti-type 2 inflammation may provide better therapeutic efficacy in refractory AD disease.

It is well known that STAT3 and STAT6 activation in keratinocytes is closely related to the pathogenesis of AD [15]. And both IL-4 and IL-13 can induce STAT6 and STAT3 activation [16]. Activation of STAT6 in keratinocytes increased the secretion of pro-inflammatory chemokines such as TSLP, IL-33 and IL-25. In contrast, STAT3 activation in keratinocytes led to the downregulation of barrier proteins such as FLG and LOR, as well as more intense itching [1]. In the present study, we found that STAT6 and STAT3 activation were enhanced by the combined action of IL-19 and IL-4/IL-13. Therefore, the combination of IL-19 and IL-4/IL-13 may enhance pathophysiological changes of keratinocytes in a STAT3- and STAT6-dependent manner. Our study also found that IL-19 mRNA was further upregulated in HaCaT cells stimulated with IL-4/IL-13 and IL-19. This enhancement further promoted their pro-inflammatory effects on keratinocytes and increased STAT3 and STAT6 phosphorylation. However, we didn't observe the upregulation of IL-4R α /IL-13R α 1 or IL-20RA/IL-20RB in HaCaT cells under the combined action of IL-19 with IL-4/IL-13. This suggests that IL-19 promotes IL-4/IL-13 induced STAT3 and STAT6 phosphorylation by acting on the signal pathway directly.

House Dust Mite (HDM) is a critical allergen in AD, initiating type 2 inflammation by activating antigen presenting cells [33]. Study also showed that allergens from house dust mites are associated with severe atopic dermatitis in children [22]. In addition, house dust mite-derived protease could induce defected skin

barrier, further aggravating the symptoms of AD [31]. In contrast, although colonization of staphylococcus aureus (*S. aureus*) in AD patients can lead to disease aggravation, the role of enterotoxin B secreted by *S. aureus* in AD is not like HDM. As with our study, we found that HDM, but not SEB, could induce IL-19 mRNA and protein expression. This finding provides further evidence about the relevance of IL-19 with the initiation and progression of AD. Future research will be directed toward illuminating the action target and underlying signal pathway by which HDM induced IL-19 production in keratinocyte.

Our study extends the insight into IL-19, a so far less explored mediator. Although it is generally accepted that IL-19 is a potentially regulatory cytokine in immune reaction, its receptor (IL-20RA) on lymphocytes has yet discovered [2]. Unlike other classic interleukins, IL-19 could also be secreted by tissue cells, including keratinocyte, and act on them [27]. It has been demonstrated that IL-19 is related to liver disease, central nervous system disorders, cardiovascular disease, inflammatory bowel disease, and arthritis [13]. Although IL-19 is rapidly increased in inflammatory conditions, it is not detected in healthy tissues. Our study further explored the effect of IL-19 on keratinocytes, and its association with Th2 cytokines (IL-4/IL-13), which not only facilitates further research about the effects of IL-19 on lymphocytes, but also promotes our understanding on the heterogeneity of AD. In the light of previous study, IL-19 may become an objective biomarker to predict AD patients' prognosis, and assist clinical management [24]. In this study, our conclusions were concluded on the basis of *in vitro* experiments. Animal models and clinical study may help dissect the role of IL-19 in AD.

In summary, although Th2-mediated immune inflammation dominates the pathogenesis of AD, other T cells, such as Th22 and Th17, and their cytokines have also been confirmed to participate in the formation of inflammatory microenvironment in AD [21]. Previous studies have shown that IL-19 was a downstream mediator of IL-17A [39], which indicated that IL-19 is closely associated with IL-17. Taken together with our study, we speculated that IL-19 may be a bridge between type 2 inflammation and IL-17A. Our research on the production and role of IL-19 presented this cytokine as an important cytokine in the pathogenesis of AD, and have important clinical significance for understanding the underlying mechanism concerning the chronic lesions formation in AD and psoriasisiform phenotypes (such as AD in Asian populations). Based on our current findings, we will further analyze the molecular mechanism of AD and develop more targeted therapies and prospective management for patients in the future.

Declarations

All authors have read and approved the content, and agree to submit for consideration for publication in the journal.

Conflict of interest: The authors have no conflict of interest to declare.

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Ethical Approval: This study does not contain any studies with human participants or animals performed by any of the authors.

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Tables

Table 1

Primer pairs used in this study.

Factor	Primer sequence(5'-3')	
	Forward	Reverse
hGAPDH	CTG GGC TAC ACT GAG CAC C	AAG TGG TCG TTG AGG GCA ATG
hIL-19	CCC TTA GAT GTG TGC TGC GTG AC	GGG TTT GGC TCC TGA TGA TCC TTG
hIL-25	TTC CTA CAG GTG GTT GCA TTO TTG G	TCA GCA GCT CCT CAG AGG TGT C
hIL-33	AGG TGA CGG TGT TGA TGG TAA GAT G	CAG AGT GTT CCT TGT TGT TGG CAT G
hTSLP	GTG CCC AGG CTA TTC GGA AAC TC	TGA AGC GAC GCC ACA ATC CTT G
hFLG	CCA TCA CAG CCA CAC CAC ATC C	GTG CCG TCT CCT GAT TGT TCC TC
hLOR	TTA CTC CTC TCA GCA GAC CAG TCA G	CCT CCA CAG CTA CCA CCT CCT C
hKeratin-10	TTT GGT GGA TTT GGA TTT GGA GGA GAT C	AAG CCA GGC GGT CAT TCA GAT TC
hIL-20RA	TGG AGC CGA ACA CTC TTT ACT	CGG GCA AAA CAT ACC AGA AGA TG
hIL-20RB	TCC ACC TGG TTA TTG AGC TGG	CCC ACT CCT CAC CAT TTT GAC A

h: human; GAPDH: glyceraldehyde phosphate dehydrogenase gene; IL: interleukin; TSLP: thymic stromal lymphopoietin; FLG: filaggrin; LOR: loricrin R: receptor

Figures

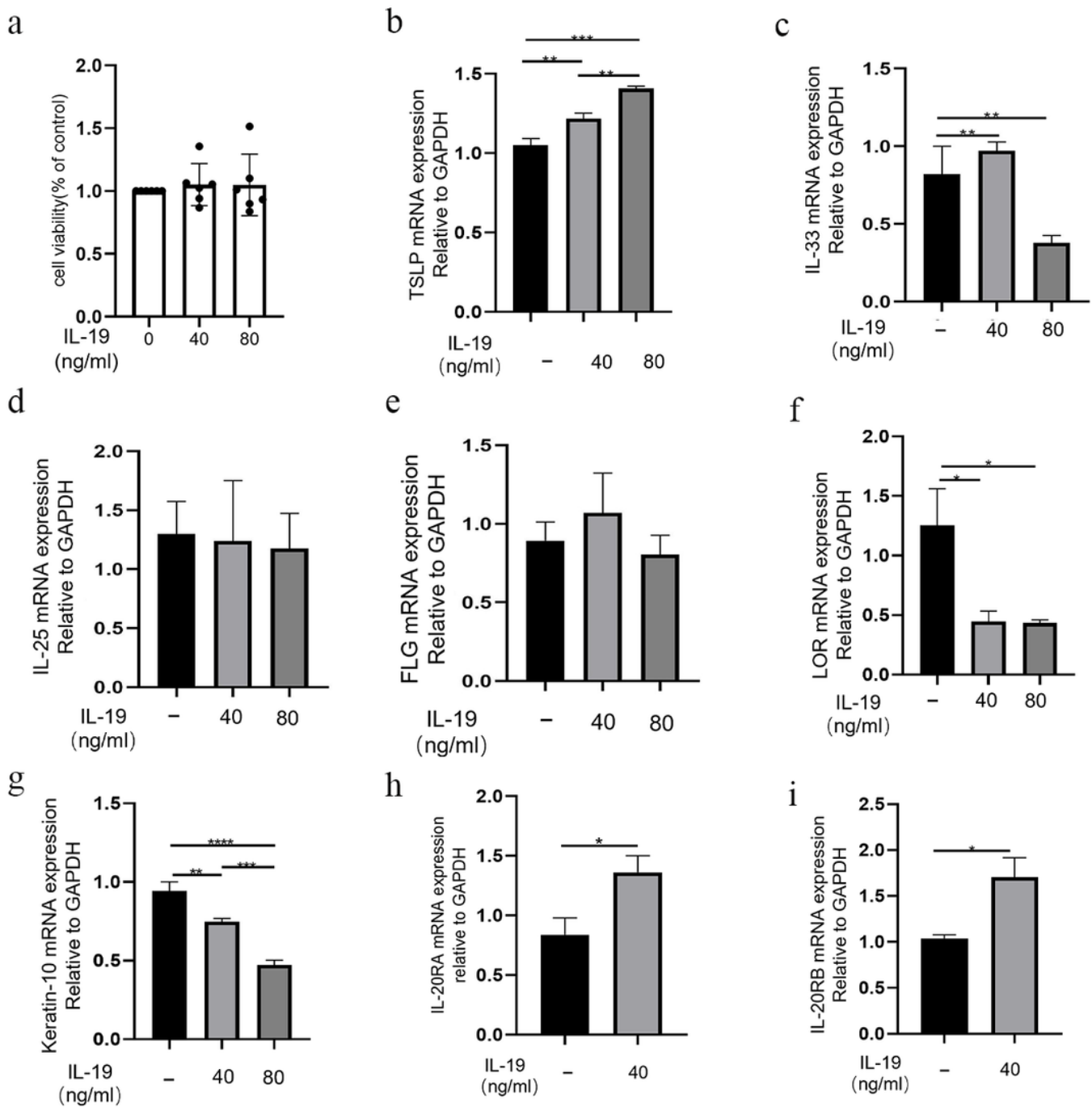


Figure 1

Partly regulatory effects of IL-19 on keratinocytes by binding to IL-20RA/RB.

(a-i) HaCaT cells were serum starved, then stimulated with 40ng/ml, 80ng/ml IL-19 for 24 h. (a) The vitality of HaCaT cells by CCK-8 assay. (b-g) Expression of (b) TSLP mRNA, (c) IL-33 mRNA, (d) IL-25 mRNA, (e) FLG mRNA, (f) LOR mRNA, (g) keratin-10 mRNA were measured. (h, i) Quantification of IL-20RA/RB mRNA in HaCaT cells when stimulated with 40ng/ml IL-19. Relative mRNA expression was

normalized to GAPDH. Data are expressed as mean \pm S.E. of three independent experiments. * P <0.05 ** P <0.01 *** P <0.001 **** P <0.0001 by one-way non-parametric ANOVA. FLG, Filaggrin; LOR, loricrin; TSLP, Thymic stromal lymphopoietin; S.E., standard error.

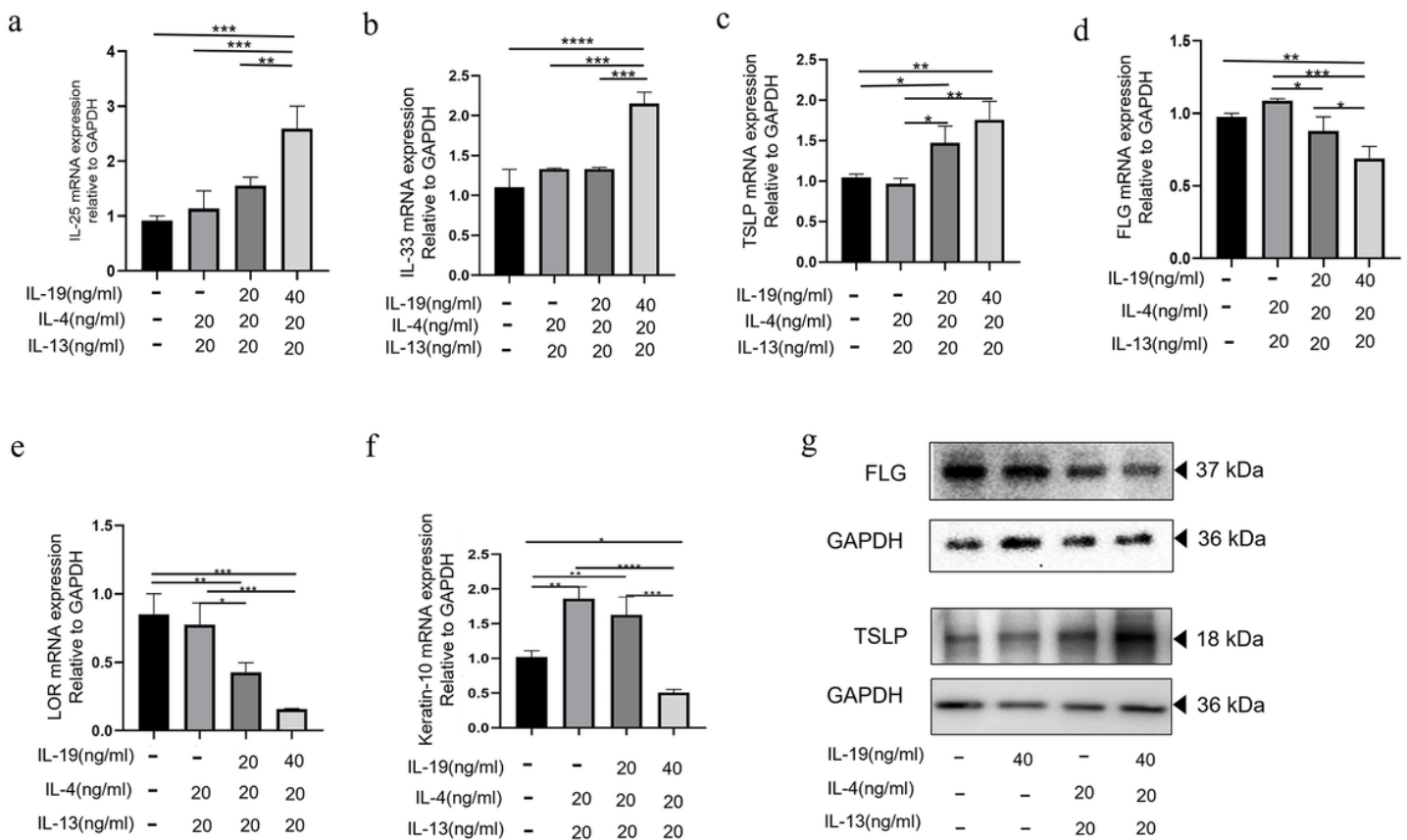


Figure 2

IL-19 strengthened IL-4/IL-13-induced keratinocyte effects.

(a-f) After serum starvation, HaCaT cells were treated with different cytokines for 24 hours. Then RNA was extracted and target mRNA level was determined by quantitative RT-PCR normalized to GAPDH, (a)IL-25, (b)IL-33, (c)TSLP, (d)FLG, (e)LOR, (f)keratin-10. (g) The protein of FLG and TSLP were measured in HaCaT cells after stimulation by IL-19 or IL-4/IL-13 alone, or both in combination for 24 h. All values are represented as mean \pm S.E. normalized as % of control group. Significant differences from the IL-19 plus IL-4/IL-13 group are presented. ** P <0.01 *** P <0.001 **** P <0.0001 by one-way non-parametric ANOVA. All results shown are from at least 3 independent experiments, with identical results.

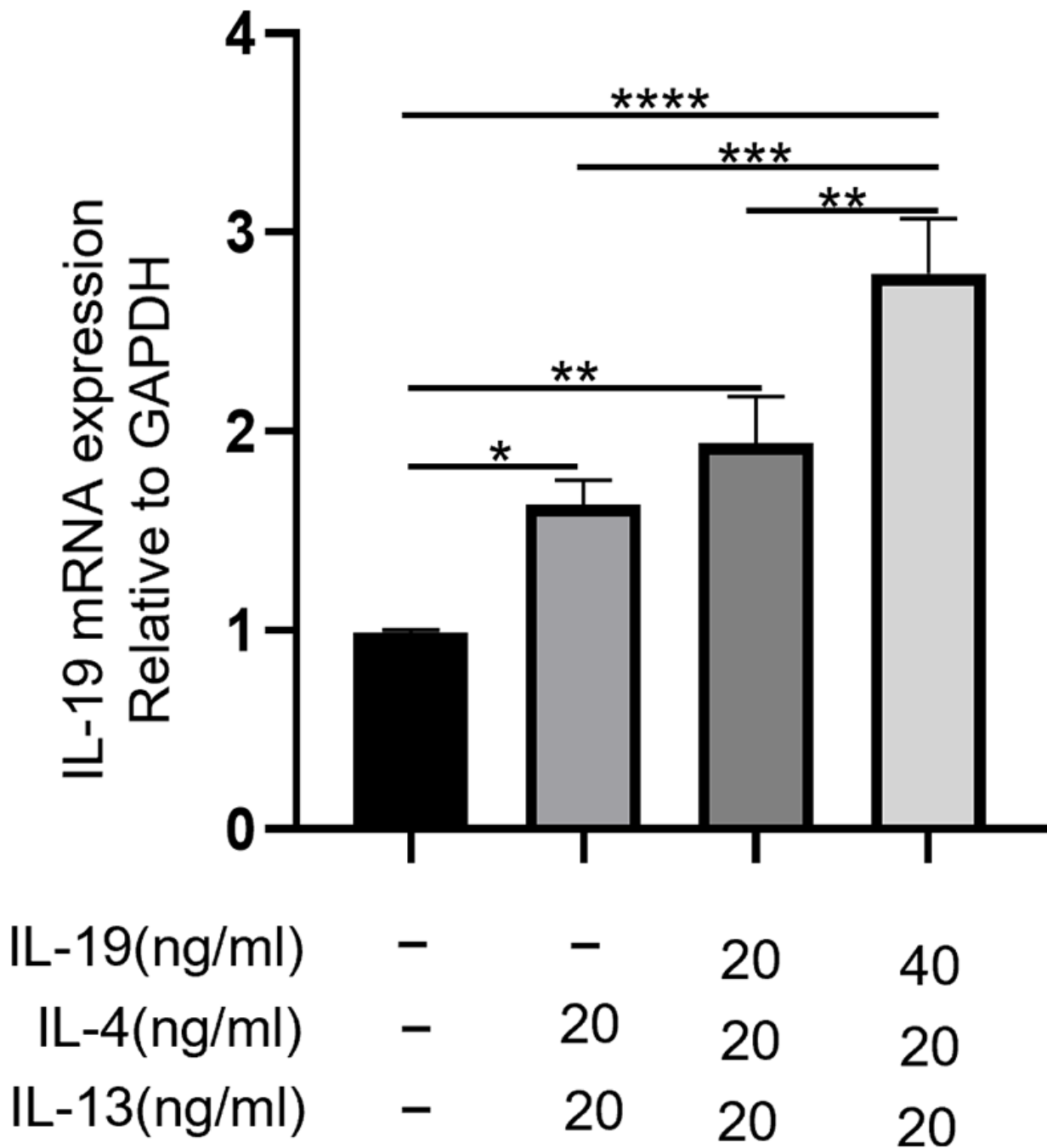


Figure 3

Facilitative effect of IL-4/IL-13 with IL-19 regarding IL-19 mRNA expression in HaCaT cells. IL-19 mRNA was measured in HaCaT cells stimulated with 20ng/ml IL-4/IL-13 with or without 20ng/ml, 40ng/ml IL-19. Data are expressed as mean \pm S.E. **P<0.01 ***P<0.001 ****P<0.0001 by one-way non-parametric ANOVA. All results shown are from at least 3 independent experiments, with identical results.

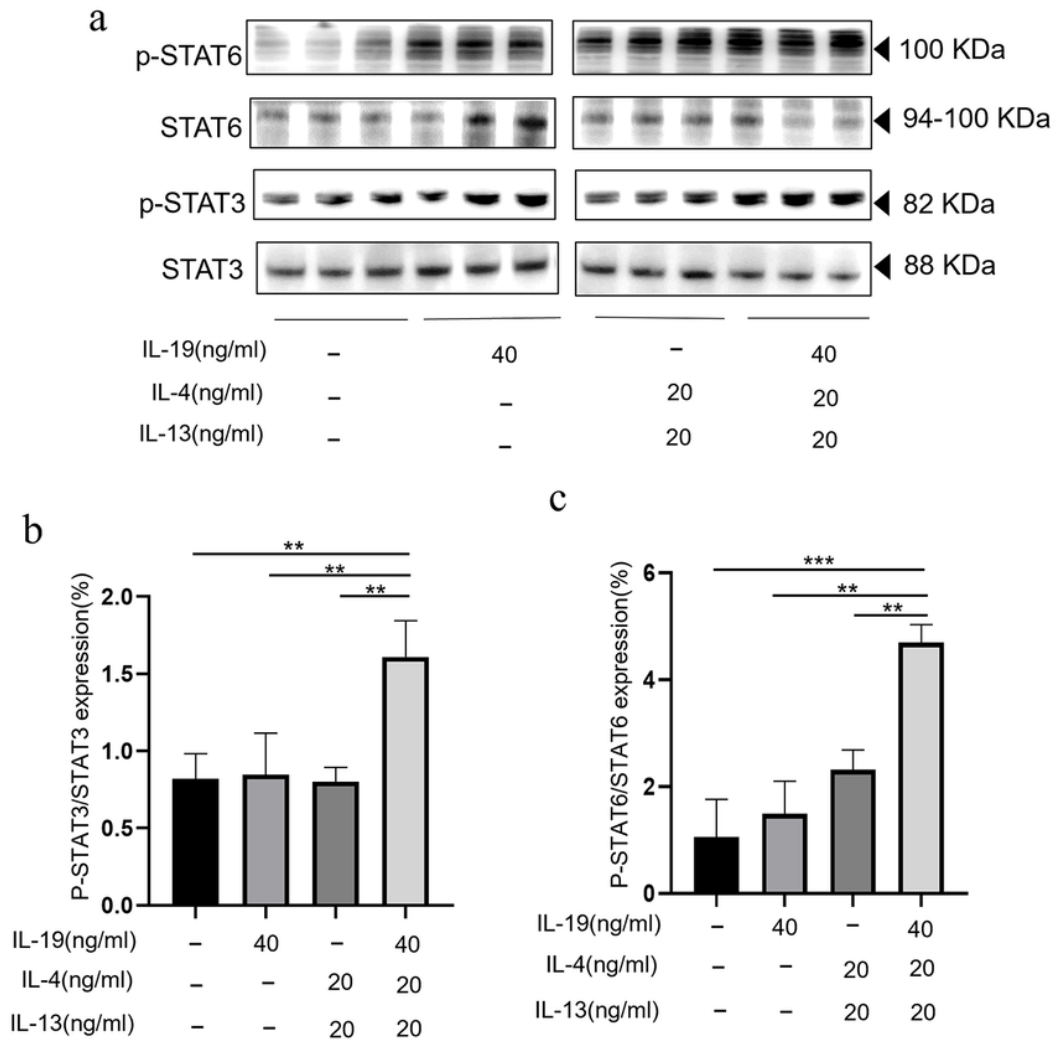


Figure 4

Effects of IL-19 with IL-4/IL-13 on STAT3 and STAT6 phosphorylation in HaCaT cells.

The cells were stimulated with IL-4/IL-13, IL-19 and IL-4/IL-13 plus IL-19 for 30 min. Levels of phosphorylation of STAT3 and STAT6 were evaluated by western blot using the different antibodies. (a, b) STAT3 phosphorylation in HaCaT cells. (c, d) STAT6 phosphorylation in HaCaT cells. Blots were measured with densitometry, and normalized to total protein. Bars quantified the densities of bands. Relative protein level was computed based on indicated ratios. Data are expressed as mean \pm S.E. of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ STAT-3/6, signal transducer and activator of transcription 3/6; S.E., standard error.

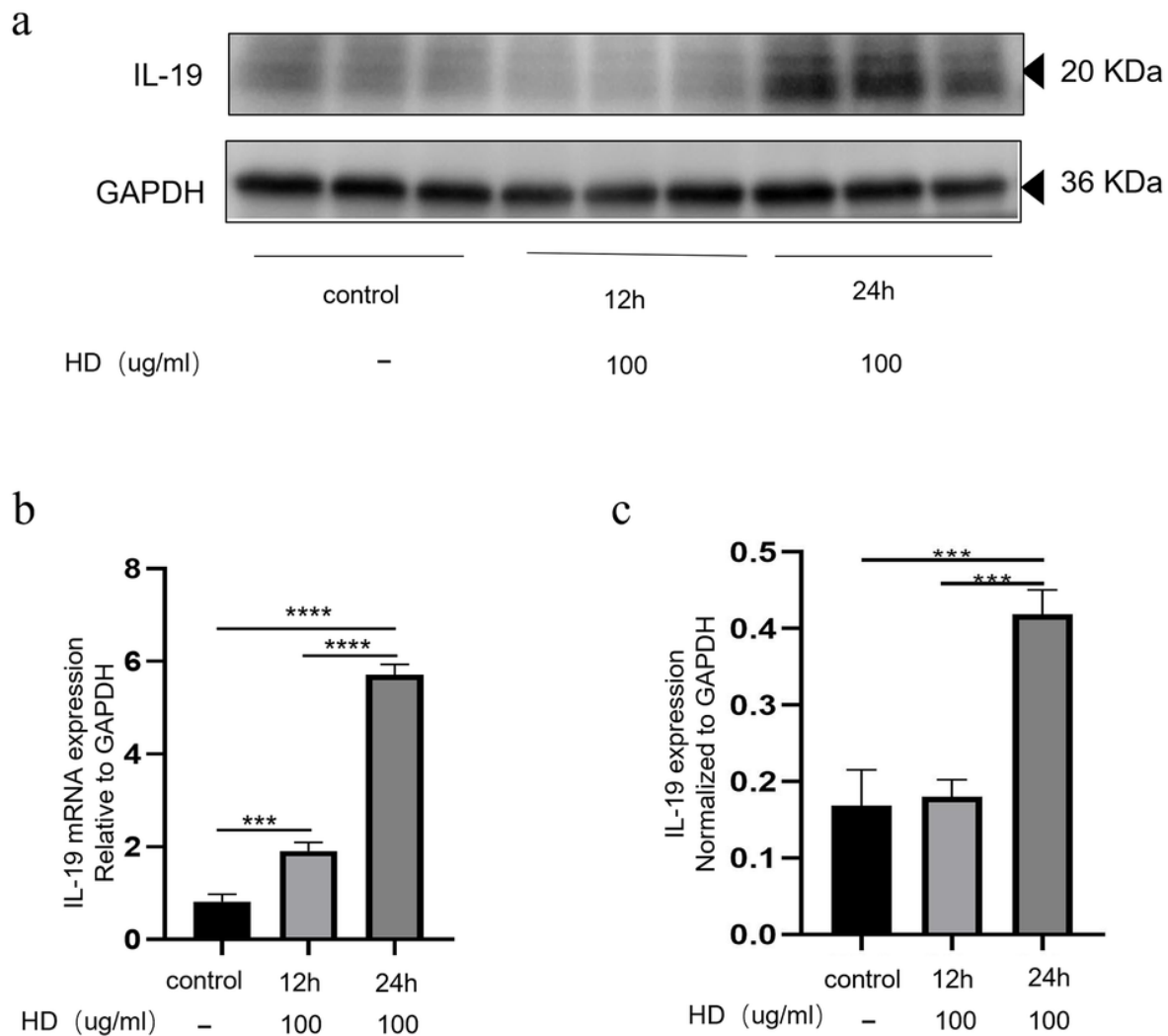


Figure 5

House dust mite strengthens IL-19 protein and mRNA abundance in HaCaT cells.

(a) HaCaT cells were serum-starved, then treated with 100ug/ml HDM for 12h, 24h. IL-19 expression was determined by western blot. (b) HaCaT cells were serum-starved, then stimulated with 100ug/ml HDM. RT-PCR analysis for the mRNA of IL-19 in HDM (100ug/ml)-stimulated keratinocytes for different time courses. Data are represented as mean \pm S.E. *** $P < 0.001$ **** $P < 0.0001$ by one-way non-parametric ANOVA. All results shown are from at least 3 independent experiments, with identical results.

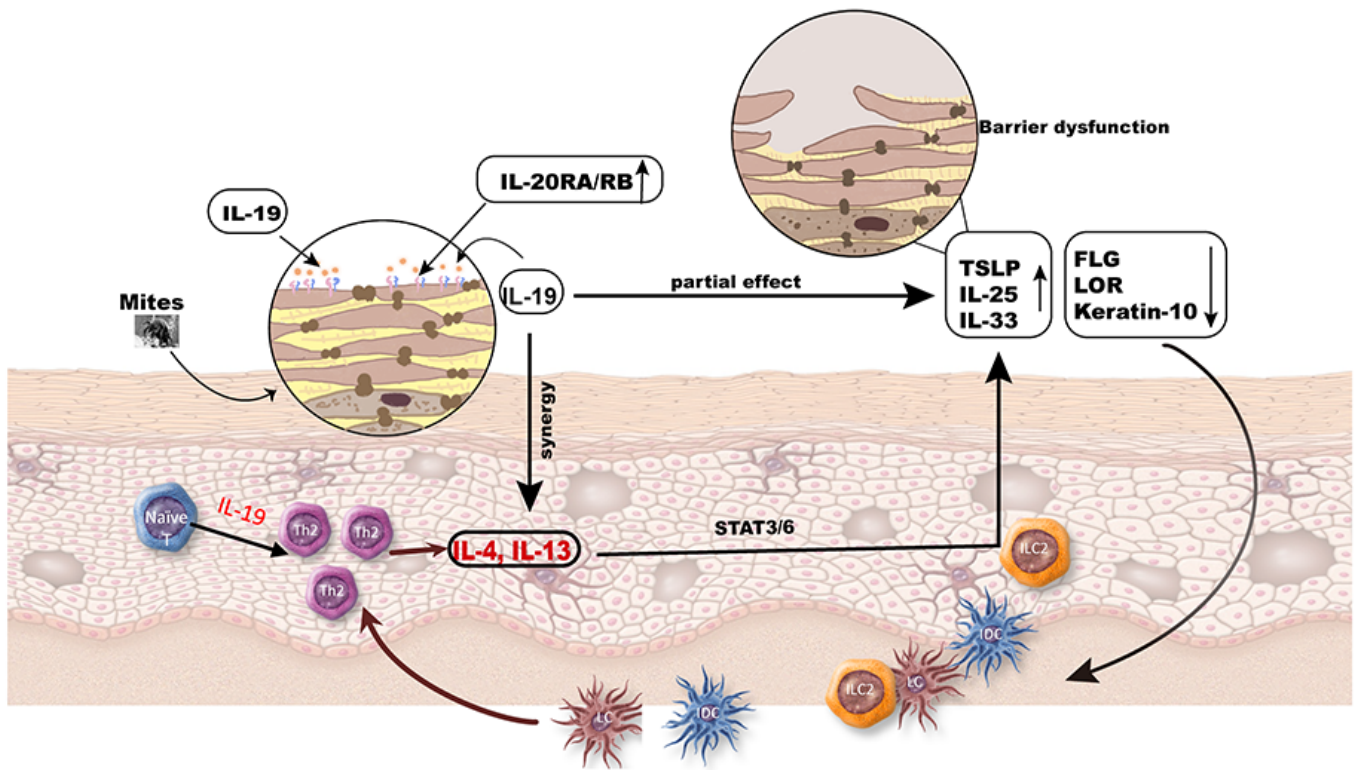


Figure 6

Schematic diagram of the overall research analysis.

House Dust Mite stimulated the production of IL-19 in keratinocytes. IL-19 induced its receptors expression in keratinocytes, and further exerted partial effects on keratinocytes. IL-19, with IL-4/IL-13 exaggerated the pathology of keratinocytes, including downregulating barrier proteins (FLG, LOR and keratin-10) and upregulating epithelium-derived cytokines (TSLP, IL-25 and IL-33). This effect is achieved by activating STAT3/6. IL, interleukin; TSLP, thymic stromal lymphopoietin; DC, dendritic cell; JAK, Janus Kinase; STAT, signal transducer and activator of transcription; FLG, Filaggrin; LOR, loricrin; AD, atopic dermatitis.