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IL-13-Amphiregulin pathway exacerbates itch in allergic contact dermatitis

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Running title

IL-13–AREG Signalling Exacerbates Itch

To the Editor

Itch is an instinctive sensory response that provokes scratching and serves as a protective mechanism against potential threats. It is a pervasive and distressing symptom across many dermatological and systemic disorders, particularly atopic dermatitis (AD) and allergic contact dermatitis (ACD), each of which affects nearly 20% of the population. Persistent itch disrupts the skin barrier, fuelling disease chronicity through a self-perpetuating itch–scratch cycle that worsens lesions and diminishes quality of life. The underlying mechanisms remain largely unknown.

Amphiregulin (AREG), recently implicated in chronic pain is a member of the epidermal growth factor (EGF) family and a type 2 cytokine–associated protein that binds to the epidermal growth factor receptor (EGFR) to regulate cell proliferation, differentiation, and migration (Zaiss et al., 2015). Beyond epithelial cells, AREG is also produced by mast cells, basophils, type 2 innate lymphoid cells (ILC2s), and regulatory CD4⁺ T cells (Zaiss et al., 2015). Initially expressed as a membrane-anchored precursor protein, AREG is cleaved by ADAM17 (a disintegrin and metalloprotease 17), facilitating EGFR signalling that contributes to inflammation and tissue repair. AREG has been implicated in multiple inflammatory disorders, including psoriasis, arthritis, and AD, by promoting inflammatory cytokine expression, keratinocyte proliferation, and epidermal remodelling. However, the role of AREG dysregulation in itch pathogenesis remains undefined. This study aims to elucidate the role of AREG signalling in skin upon T_H2 cytokine stimulation and the resulting alterations in neurocutaneous communication in both acute and chronic itch models. We will also evaluate the effect of an anti-AREG neutralising antibody on itch generation.

To investigate whether *Areg* expression is upregulated in chronic itch conditions, bulk transcriptomic (RNA-seq) data from the lesional skin of three different chronic itch models were analysed: (i) the MC903-induced AD-like cheek model, (ii) the 2,4-dinitrofluorobenzene (DNFB)-induced ACD ear model, (iii) the acetone/ether (1:1) mixture followed by water treatment (AEW)–induced dry skin (xerosis) cheek model. Bulk transcriptomic analysis of skin biopsies, quantified as fragments per kilobase of transcript per million mapped reads (FPKM), showed significant *Areg* upregulation across all three models compared with their respective vehicle-treated controls. *Areg* expression was the highest in the MC903-induced AD-like ear skin, intermediate in DNFB-treated skin, and lowest in the AEW-induced xerosis model.

(**Figure 1a**). Moreover, AREG immunoreactivity in the epidermis was prominent in both ACD and AD lesions but not in AEW lesions (**Figure 1b**), suggesting that epidermal AREG may play a distinct role in the pathological progression of these conditions. To investigate cell-specific *AREG* expression in human AD patients, we interrogated a publicly available single-cell transcriptomic dataset from GSE153760 (Rojahn et al., 2020). UMAP visualisation of skin cell clusters was generated using Harmony-corrected dimensions (umapharmony). *AREG* expression was distributed across several clusters, including keratinocyte subsets such as suprabasal keratinocytes 1/2/3 and basal keratinocytes, as well as leukocyte clusters such as cDC2, $\gamma\delta$ T cells, T cells cluster 3, mDC, Langerhans cells, monocytes, pDC, and mast/basophils (**Figure 1c**). *AREG* expression was predominantly increased in leukocyte clusters in lesional AD skin, including cDC2, mDC, monocytes, pDC, and one T cell cluster (T cells 3). While suprabasal keratinocyte cluster 1 initially showed no difference in *AREG* expression between lesional AD and healthy skin controls, subclustering revealed *AREG* expression is induced in subclusters 4/7 in lesional AD skin (**Supplementary Figure S1, a-c**).

To determine whether AREG upregulation is also associated with specific keratinocyte populations in ACD, we examined a published DNFB-induced ACD mouse model (Liu et al., 2024). However, available single-cell transcriptomic datasets for ACD remain limited, and in this dataset, keratinocyte clusters were not identified because they were poorly represented. Therefore, whether AREG upregulation in ACD is associated with specific keratinocyte subsets remains to be determined and requires further investigation.

To explore a potential link between AREG and IL-13, a pruriginous T_H2 cytokine reported to be upregulated in both AD and ACD at cutaneous and systemic levels (Tsoi et al., 2019, Ungar et al., 2017, Neis et al., 2006), bulk transcriptomic data from our murine models of chronic itch were analysed. *Il13* transcription was increased in whole-skin biopsies from the ACD model compared with the AD model, whereas no changes were observed in the AEW model (**Figure 1d**). However, because *Areg* expression is regulated by multiple pathways and bulk RNA-sequencing captures the combined transcriptional profiles of heterogeneous cell populations, whole-skin *Il13* levels alone may not fully explain the observed *Areg* expression patterns. Although IL-13 has been shown to directly induce *Areg* expression in keratinocytes, the elevated *Areg* expression observed in MC903-treated skin likely reflects the integrated effects of IL-13 signalling, epithelial activation, and additional regulatory pathways.

To directly assess whether IL-13 regulates *AREG* expression in keratinocytes, we treated normal human epidermal keratinocytes (NHEKs) with IL-13 and performed bulk transcriptomic analysis. IL-13 treatment significantly enhanced *AREG* transcription relative to vehicle-treated controls (**Figure 1e**), supporting a mechanistic link between Th2 cytokine signalling and keratinocyte activation via *AREG* induction. IL-13 appears to induce *AREG* transcription in a relatively specific manner, rather than as a general consequence of type-2 cytokine stimulation. Consistent with this, IL-4 has been reported to fail to induce *AREG* production in keratinocytes (Serezani et al., 2017) (**Supplementary Table S1**). The responsiveness of primary murine keratinocytes to *AREG* was further assessed by calcium imaging at a physiologically relevant concentration (300 ng/mL), as previously reported (Morimoto et al., 2018). Approximately 31.5% of total keratinocytes exhibited calcium transients in response to *AREG* stimulation, which could be categorised into three subpopulations based on response kinetics: fast (**Figure 1f, left**), intermediate (**Figure 1f, middle**), and slow (**Figure 1f, right**), revealing functional heterogeneity and supporting a paracrine role for *AREG* in epidermal signalling.

To investigate the downstream signalling events of *AREG*, a cytokine antibody array revealed that *AREG*-activated keratinocytes released a broad spectrum of cytokines, including Serpin E1, IL-3, -4, -5, HGF (Hepatocyte Growth Factor), EGF (Epidermal Growth Factor), CD105 (Endoglin), IL-27P28 (the alpha subunit of the cytokine interleukin-27), IL-28A, OPN(ETA-1), PD-EGCF, RESISTIN, and CD106 (**Figure 1g**). Consistently, transcriptomic analysis demonstrated upregulation of *TRPV3*, *SERPINE1*, and *TSLP* (**Figure 1h**), which are critical itch modulators in epidermis. In contrast, parallel experiments with cultured murine dorsal root ganglion (DRG) and trigeminal ganglion (TGN) neurons did not reveal detectable responses to *AREG* stimulation at comparable physiological concentrations. Given that several of these factors are implicated in pruritus (Larkin et al., 2021), these findings suggest that murine keratinocytes not only secrete *AREG* but also respond to it in an autocrine manner, thereby promoting the release of itch-associated cytokines. Moreover, our experiment also showed that pharmacological inhibition of EGFR by Gefitinib attenuated *AREG*-induced *TRPV3* transcription, supporting an EGFR-dependent mechanism (**Figure 1i**).

We then evaluated the transient itch-inducing effect of *AREG* using the mouse cheek model and subsequently analysed downstream transcriptional changes in the skin. Intradermal injection of *AREG* elicited scratching behaviour compared with vehicle-treated controls

(**Figure 2a–c**), with comparable scratching bout frequencies across doses. Time-course analysis revealed that AREG did not evoke an immediate itch response; instead, peak scratching activity occurred 20–30 minutes after injection (**Figure 2d**). RNA-seq and heatmap analysis of skin biopsies from AREG-injected skin demonstrated upregulation of multiple genes, including *Krt17*, *Nppb* [gene encoding brain natriuretic peptide (BNP)], *Klk6* (kallikrein-6), *Tgf alpha* (*Transforming Growth Factor alpha*), *Krt6a* (Keratin 6A), *Mmp1b* (matrix metalloproteinase-1b), *Krt16* (Keratin 16), and *Mmp1a* (Matrix Metalloproteinase 1a) (**Figure 2d, f**). Collectively, these results identify AREG as an itch inducer in mice and suggest that it promotes the expression of itch-associated neuropeptides and epidermal remodelling genes in the skin.

To validate the role of AREG in AD- and ACD-associated itch and its potential as a therapeutic target, an intraperitoneal injection of a neutralising anti-AREG antibody was administered to assess the effect of AREG inhibition on pruritic behaviour in both the MC903-induced and DNFB-induced models. In the DNFB model, antibody treatment from day 2 to day 9 significantly attenuated scratching behaviour on day 7 compared with vehicle-treated controls (**Figure 2g, h, i**). In contrast, no significant reduction in scratching was observed in the MC903 model, although a modest trend toward decreased itch behaviour was noted (**Figure 2j, k**).

Taken together, IL-13 induces epidermal *Areg* expression, which in turn upregulates the *Trpv3* and promotes the synthesis of pruritogenic mediators, including *Serpin E1* and *Tslp*, thereby promoting pruritus. AREG further exacerbates itch by enhancing the expression of the key pruritogenic neuropeptide *Nppb* in the skin (Dai et al., 2026). Consistently, pharmacological blockade of AREG significantly attenuates pruritic responses in the ACD model. Unlike AD, ACD lacks targeted biologic therapies and is mainly managed by allergen avoidance and broad anti-inflammatory treatments. Our findings identify AREG as a potential therapeutic target for ACD. Although no anti-AREG therapies are currently approved, targeting AREG translation may represent a novel strategy to reduce AREG production and suppress both autocrine and paracrine AREG–EGFR signalling. Our experiment also revealed that AREG stimulation significantly increased β -hexosaminidase (β -Hex) release from primary mouse mast cells, indicating enhanced mast cell degranulation (**Supplementary Figure S1, d**). Whether direct activation of mast cells or T cells through AREG–EGFR signalling is sufficient to induce delayed pruritus remains unknown and warrants further investigation.

Ethics Statement

All animal procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Henan University and were approved by the Henan University Animal Ethics Committee.

Data Availability Statement

All data generated or analysed during this study are included in this manuscript. RNA-seq datasets related to this article can be found at <https://www.ncbi.nlm.nih.gov/gds/>. These include AREG-injected vs control cheek skin data (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE232421>), RNA-seq datasets related to IL-13 treatment of NHEK cells (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE172006>), mouse tissue samples from ACD models (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE237038>), mouse tissue samples from AEW models (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE237113>), and cheek skin from MC903 models (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE230812>).

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Conflict of Interest

The authors state no conflict of interest.

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Author Contributions

Conceptualization: JM and JW. Data curation and formal analysis: XD, XY, RL, WC, MK, RZ, OW and SK. Resources: OW and WC. Writing-original draft: JM, JW, XD. Funding acquisition: JM and WC. Writing-review & editing: All authors.

Declaration of AI or LLMs use

No AI or LLM tools were used to prepare this manuscript.

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Figure legends

Figure 1. AREG is upregulated in pruritic skin; IL-13 induces AREG in keratinocytes; AREG activates keratinocytes to enhance itch-related mediators.

a. RNA-seq comparison of *Areg* transcript levels (FPKM) in murine skin from MC903-induced AD-like, DNFB-induced ACD, and AEW-induced dry skin models (n = 3–5 mice per group). GEO accession: GSE230812 (MC903), GSE237038 (DNFB), GSE237113 (AEW).

b. Representative images showing *Areg* expression in murine skin from MC903-induced AD-like, DNFB-induced ACD, and AEW-induced dry skin models. Breaking lines indicate epidermal-dermal junctions. Scale=50 μ m.

c. Cell-specific expression of *AREG* in human AD skin (GEO accession: GSE153760(Rojahn et al., 2020)). Upper panel: Harmony UMAP reveals 21 unique clusters in healthy and AD human skin; Lower panel: Feature plot of AREG expression in LAD skin and healthy control skin samples. Dataset includes 15 participants divided into two groups: 8 individuals with AD and 7 HC. AD group consists of 5 males and 3 females, with ages ranging from 18 to 42 years, and EASI ranging from 5.5 to 46.5. The HC group includes 3 males and 4 females, with ages ranging from 27 to 49 years(Rojahn et al., 2020).

d. RNA-seq comparison of *Il13* transcript levels (FPKM) in murine skin from MC903-induced AD-like, DNFB-induced ACD, and AEW-induced dry skin models (n = 3–5 per group).

e. RNA-seq analysis of AREG transcripts (FPKM) in IL-13–treated NHEKs versus vehicle-treated controls (n = 3). GEO accession: GSE172006. RT-qPCR confirmed this upregulation (n=3).

f. Calcium imaging of mouse keratinocytes following Areg treatment: temporal subpopulation analysis with each dot representing a single cell response; representative calcium traces and responder profiles (F/F₀ over time) comparing Areg versus vehicle treatment for each cluster. The number of cells analyzed is shown above the Venn diagram.

g. Fold change (FCH) of mediator release in cultured murine primary keratinocytes following Areg treatment relative to vehicle controls.

h. Comparison of *TRPV3*, *SERPIN E1*, and *TSLP* transcript levels in Areg-treated versus vehicle-treated cultured murine primary keratinocytes (n = 3).

i. Gefitinib inhibited AREG-induced *TRPV3* transcription in cultured HaCaT cells (n=3).

Data are presented as mean $\pm$ SEM. Statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001; Student's unpaired t-test with Welch's correction.

Figure 2. Areg induces site-specific acute scratching in the mouse cheek, promotes itch-related neuropeptides, and Areg neutralisation alleviates ACD-associated itch.

a–d. Effect of intradermal injection of vehicle or varying doses of AREG on scratching behaviour in mice: total scratching bouts (**a–c**) and time course of itch-like responses (**d**) (n = 8 per group).

e, f. RNA-seq and heatmap analysis of transcripts in AREG-injected versus vehicle-injected cheek skin (n = 3 per group). GEO accession: GSE232421. For **e**, *** represents FDR<0.001.

g–i. Experimental protocol for AREG neutralisation in DNFB-induced ACD: schematic of antibody or vehicle (PBS) intraperitoneal injections (red arrows indicate injection days) (**g**), daily spontaneous scratching bouts recorded over 60 min (**h**), and comparison of scratching bouts on day 1 and day 7 (**i**). (n = 5 per group)

j–k. Experimental protocol for AREG neutralisation in MC903-induced AD: schematic of antibody or vehicle injections (**j**), daily spontaneous scratching bouts recorded over 60 min (**k**). (n=5 per group).

For RNA-seq in panel e, FDR < 0.001 (***). For all other panels, data are presented as mean $\pm$ SEM; ns, P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; for others, Student's unpaired t-test with Welch's correction were applied.



