JOURNAL OF CUTANEOUS IMMUNOLOGY AND ALLERGY

TYPE Letter to the Editor
PUBLISHED 20 November 2025
DOI 10.3389/jcia.2025.15510

Check for updates

OPEN ACCESS

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RECEIVED 30 August 2025
REVISED 09 October 2025
ACCEPTED 27 October 2025
PUBLISHED 20 November 2025

CITATION
Nishimura M, Matsushima Y, Imai Y and Yamanaka K (2025) Temporal dynamics of cytokine production balance between innate and adaptive lymphocytes in atopic dermatitis model mice.
J. Cutan. Immunol. Allergy 8:15510.
doi: 10.3389/jcia.2025.15510

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Temporal dynamics of cytokine production balance between innate and adaptive lymphocytes in atopic dermatitis model mice

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KEYWORDS

atopic dermatitis, innate lymphoid cells, helper T lymphocytes, cytokine, type 2 inflammation

Dear Editors,

Atopic dermatitis (AD) involves a complex interplay between innate and adaptive immune responses. In the initial phase of disease onset, innate immunity predominates. Activated group 2 innate lymphoid cells (ILC2s) release type 2 cytokines, including IL-13, IL-5, and IL-4, which further disrupt the skin barrier, facilitating the entry of various antigens into the epidermis and thereby promoting the induction of antigen-specific effector T helper (Th) 2 cells. This process represents a transition from innate to adaptive immunity. Previous studies have examined the changes in mRNA expression of various cytokines during the acute and chronic phases of human AD and investigated the presence of ILCs, cytokines, and cytokine receptors in human AD (Supplementary Material S1). Although previous murine studies have compared the numerical balance of cytokine-producing ILCs and Th cells [1], no reports have tracked their temporal dynamics during the progression of dermatitis.

Here, using three distinct spontaneous AD model mouse strains, we longitudinally profiled cytokine production by ILCs and Th cells in skin-infiltrating lymphocytes, skin-draining cervical lymph nodes, and spleens at disease onset, the acute phase, and the chronic phase. As AD models, keratin 14 promoter-driven IL-33-overexpressing transgenic (IL-33Tg): IL-33-induced acute-phase dermatitis [2], keratin 14 promoter-driven IL-18-overexpressing transgenic (IL-18Tg): models of chronic-phase AD [3], keratin 14 promoter-driven caspase-1-overexpression transgenic (KCASP1Tg) mice, which are regarded as combined features of both AD and psoriasis histologically and immune profiles [4], were used. 2-, 4-, and 6-month-old female transgenic for IL-33Tg and KCASP1Tg mice were used. For IL-18Tg, 4-, 6-, and 8-month-old mice were used, as dermatitis typically develops late in life. The Mie University Board Committee approved the experimental protocol for Animal Care and Use (#22-39-7).

Purified mononuclear cells were cultured in the presence of Phorbol Myristate Acetate (25 ng/mL), ionomycin (1 μg/mL), and brefeldin A (1 g/mL) for 4 h, and then LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to exclude apoptotic and necrotic cells. The cultured mononuclear cells

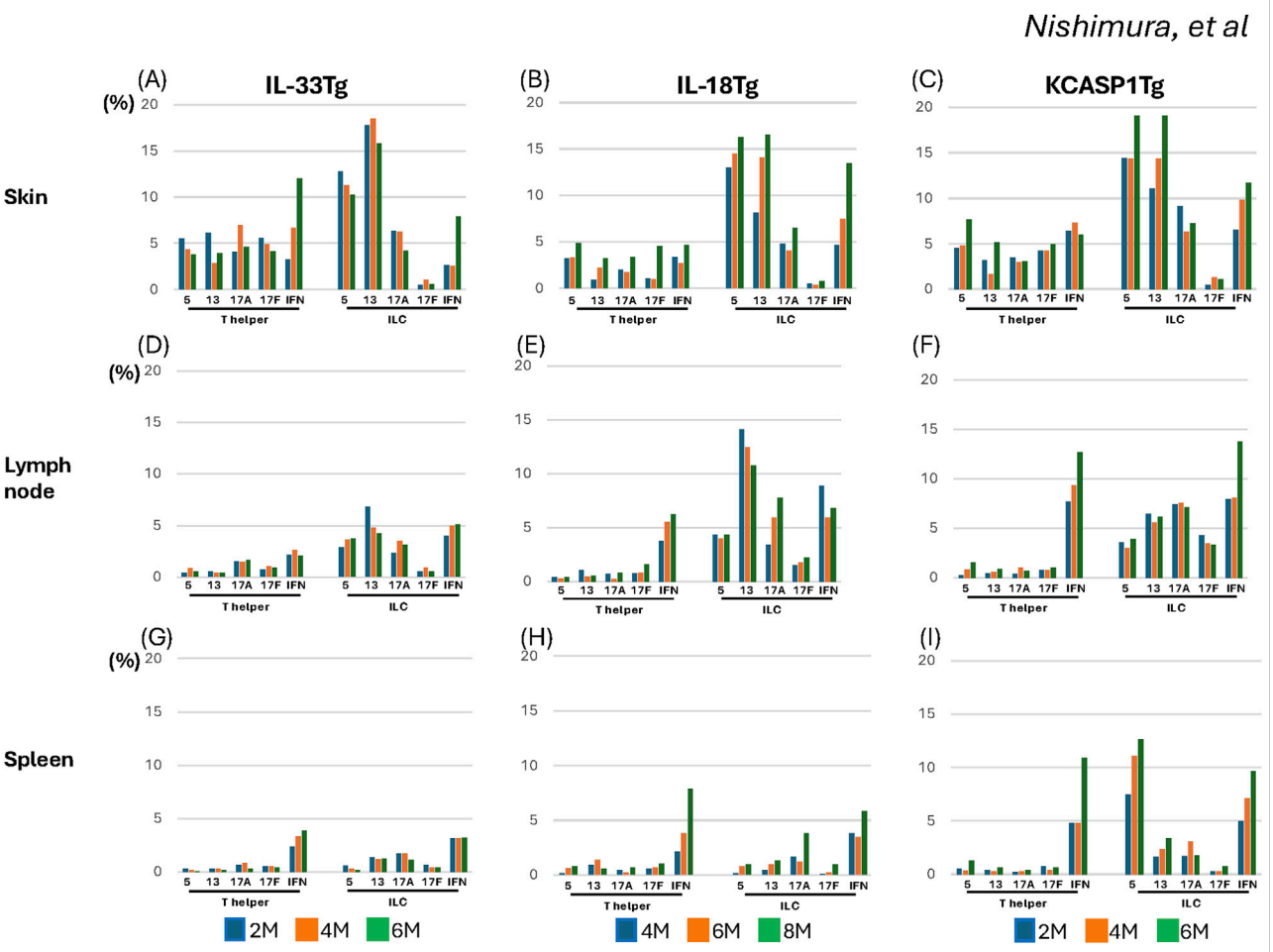

FIGURE 1
Cytokine production by innate lymphoid cells (ILCs) and T helper (Th) cells was evaluated in skin-infiltrating lymphocytes, skin-draining cervical lymph nodes, and spleens at disease onset, acute, and chronic phases in three atopic dermatitis (AD) model mouse strains: IL-33Tg, IL-18Tg, and KCASP1Tg (N = 5 each). Female IL-33Tg and KCASP1Tg mice aged 2, 4, and 6 months (2M, 4M, 6M) were analyzed, whereas IL-18Tg mice aged 4, 6, and 8 months (4M, 6M, 8M) were used because of their later disease onset. Cytokine expression (IL-13, IL-5, IL-17A, IL-17F, and IFN-γ) was assessed in $CD3^+$, $CD4^+$, and $CD8a^+$ T cells by flow cytometry to characterize Th cell responses. Mononuclear cells were also gated for ILCs by excluding populations positive for lineage-specific surface markers, and cytokine expression within ILCs was quantified. Data were expressed as the percentage of cytokine-producing cells relative to the total ILC or Th cell population. Bar graphs illustrate data on immune cells across three tissues: skin, lymph node, and spleen. Each row corresponds to a different tissue, and each column represents IL-33Tg, IL-18Tg, and KCASP1Tg. The graphs show immune cell percentages in T helper and ILC categories across ages two, four, and six months for IL-33Tg and KCASP1Tg, and four, six, and eight months for IL-18Tg with varying color codes. Skin-infiltrating lymphocytes **(A–C)** ILCs predominantly produced IL-13 and IL-5 throughout the onset, acute, and chronic phases in all three AD models. IL-17A was detected at lower levels, whereas IL-17F was virtually absent. IFN-γ production by ILCs was first observed during the acute phase and progressively increased with disease progression. In Th cells, all cytokines were produced at comparable frequencies without clear temporal changes across strains, except for an increase in IFN-γ–producing Th cells during the chronic phase in IL-33Tg mice. Skin-draining lymph nodes **(D–F)** ILCs produced IL-13 and IL-5, along with substantial IFN-γ and detectable 1L-17A, whereas IL-17F remained low. Th cells in lymph nodes produced IFN-γ but exhibited generally weak cytokine production overall, with no significant temporal shifts in cytokine balance. Spleen **(G–I)** Both ILCs and Th cells included a high proportion of IFN-γ–producing cells. Splenic ILCs from some mice also produced IL-17A and IL-5, although these findings varied among individuals. Splenic Th cells, by contrast, consistently displayed uniformly low cytokine production. The cytokines were abbreviated as follows: IL-5; 5, IL-13; 13, IL-17A; 17A, IL-17F; 17F, IFN-γ; IFN.

were stained with the following surface antibodies: CD45-APC, CD3-PE-Cy7, CD4-PE-Cy7, CD8a-PE-Cy7, CD45R-PerCp-Cy5.5, CD3-PerCp-Cy5.5, CD4-PerCp-Cy5.5, FceRI- PerCp-Cy5.5, CD8a-PerCp-Cy5.5, Ly-6G and Ly-6C-PerCp-Cy5.5, Siglec-F-PerCp-Cy5.5, TCRγδT-PerCp-Cy5.5 (BioLegend, San Diego, CA, USA) in cell surface staining buffer containing 0.1 M PBS and 1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA), and then stained with IL-13-fluorescein isothiocyanate (FITC, BD Biosciences, Franklin Lakes, NJ, USA), IL-17A-APC-Cy7 (BD Biosciences), IL-17F-phycoerythrin (PE, BD Biosciences), Interferon-γ (IFN-γ) -Brilliant Violet 605 (BioLegend), and IL-5-Brilliant Violet 421 (BioLegend) antibodies. The expression patterns of inflammatory cytokines were measured using a BD Lyric flow

cytometer (BD Biosciences), and data were analyzed using FlowJo software (v10.10.0, Tree Star Inc., Ashland, OR, USA). As a limitation, the number of cells obtained was small, and the data is aggregated from five animals. Therefore, statistical analysis could not be performed, and this is a descriptive analysis.

Analysis of skin-infiltrating lymphocytes revealed, consistent with previous data [5], that ILCs produced a high proportion of type 2 cytokines (IL-13 and IL-5), and this profile remained consistent across onset, acute, and chronic phases in all three AD models. IL-17A was produced comparatively, and its proportion relative to other cytokines did not change over time. The production of IL-17F was substantially very low. IFN-γ production in the acute phase was detectable, whereas it showed an increasing tendency during disease evolution (Figures 1A–C).

By contrast, in skin-infiltrating Th cells, type 2, type 1, and type 3 cytokines were produced at roughly similar frequencies, and no time-dependent changes were observed across all strains. IFN-γ-producing Th cells increased in the chronic phase in IL-33Tg. Overall, the ratio of cytokine production by Th cells was lower than that of ILCs. These findings indicate that ILCs and Th cells in the skin may be subject to distinct regulatory influences, with ILCs specialized for robust cytokine secretion (Figures 1A–C).

In skin-draining lymph nodes, ILCs produced type 2 cytokines alongside substantial IFN-γ and detectable IL-17A, whereas IL-17F remained at low levels. Lymph node Th cells exhibited IFN-γ production but generally displayed a poor cytokine-producing capacity, with no specific shifts in cytokine balance over time (Figures 1D–F).

Finally, in the spleen, both ILCs and Th cells included a high fraction of IFN-γ-producing cells. Splenic ILCs from KCASP1Tg mice produced IL-5. ILCs and Th cells showed uniformly low cytokine production (Figures 1G–I). Considering the absolute number of cytokine-producing cells, Th cells are more numerous compared to those of ILCs in all mice (Supplementary Figure S1).

Due to the low number of infiltrating cells in normal skin, control analyses were not feasible. Moreover, murine immune mechanisms may not fully recapitulate those in humans, warranting the use of confirmatory studies with human samples.

In summary, lymphocytes infiltrating into the skin in AD models tend to have a higher overall rate of cytokine production, while those in the lymph nodes and spleen tend to have a lower rate. Skin-infiltrating ILCs consistently exhibit a dominant type 2 cytokine production profile. In all investigated samples, both ILCs and Th cells show a trend toward increased IFN-γ production over time. The late rise of IFN-γ likely represents a layer superimposed on a persistent type 2 milieu rather than a polarity switch. This can reinforce chronicity by licensing antigen presentation and CXCR3-ligand trafficking, while preserving type 2 dominance of ILCs. The pattern aligns with the clinical spectrum of extrinsic and intrinsic AD, suggesting consideration of IFN-γ–responsive pathways (e.g., JAK-STAT). The overall balance among type 1, type 2, and type 3 cytokines remains remarkably unchanged during disease onset, acute, and chronic phases.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by The Mie University Board Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MN conducted the investigation and wrote the draft. YM conducted the investigation. YI conducted the analysis. KY conducted the investigation and wrote the final manuscript. All authors contributed to the article and approved the submitted version.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was supported by JSPS KAKENHI Grant Number 25K11582.

Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/jcia.2025.15510/full#supplementary-material

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