

Close relationship between T helper (Th)17 and Th2 response in murine allergic contact dermatitis

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Summary

Background. Repeated exposure to allergens induces chronic allergic lesions in the skin and a shift in the cutaneous cytokine milieu to T helper (Th)2.

Aim. To assess the relationships between Th17 and Th2 response during allergic contact dermatitis (ACD) in mice.

Methods. ACD was induced in C57BL/6 mice by single or repeated epicutaneous challenge of 2,4,6-trinitro-1-chlorobenzene. Relationships between Th17 and Th2 response were analyzed by immunohistochemical observations and activity of cytokines on days 8 (first challenge), 18 (11th challenge), 28 (21st challenge) and 38 (31st challenge).

Results. On day 8, tissue levels of interleukin (IL)-17 and IL-22 were high, whereas tissue levels of IL-4 and serum IgE concentration were low. Following acute contact dermatitis, mice developed chronic eczematous lesions on day 18, and gradually improved on days 28 and 38. Tissue IL-4 and serum IgE levels corresponded to the development and improvement of chronic eczematous lesions. Numbers of Th17 cells and tissue levels of IL-17 and IL-22 rapidly decreased as IL-4 and IgE levels increased on day 18. As levels of IL-4 and IgE decreased, the number of Th17 cells and tissue levels of IL-17 and IL-22 increased again on days 28 and 38. On day 18, tissue levels of Th17 response-inducing cytokines (IL-6, IL-23 and transforming growth factor- β) were high, and IL-23-expressing cells appeared in abundance, when Th2 response was extremely high. IL-17 injection decreased tissue IL-4 and serum IgE levels.

Conclusions. Th17 correlates closely with Th2 in murine chronic ACD induced by repeated epicutaneous challenge.

Introduction

Th17 cells, a distinct lineage of effector CD4⁺ T cells, are characterized by their production of interleukin (IL)-17 and IL-22.¹ The function of Th17 cells is to defend against extracellular bacteria and fungi,^{2,3} but they are also involved in tissue destruction in autoimmunity.^{4,5} IL-17 has pleiotropic activities including induction of

tumour necrosis factor- α , IL-1 β , IL-6, IL-8, granulocyte colony-stimulating factor and monocyte chemotactic protein-1.^{6,7} IL-17 plays an important role in activating T cells in allergen-specific T-cell-mediated immune responses.⁸ IL-17 is required for the sensitization phase of 2,4,6-trinitro-1-chlorobenzene (TNCB)-induced contact hypersensitivity responses, and for Th1- and Th17-mediated immune responses.⁸ The role of IL-17 is recognized in the elicitation phase of human allergic contact dermatitis (ACD).⁹ IL-17 efficiently amplifies the allergic reaction by rendering T cells accessible to recruitment at the site of skin inflammation in ACD.¹⁰ Th17 cells produce IL-17 and IL-22, which mediate epithelial innate immunity¹¹ and inhibit IL-4 production by Th2 cells.¹² Taken together, Th17 is relatively

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well documented as playing a role in acute ACD, through the actions of its expressed cytokines, IL-17 and IL-22.

Exposure to allergens on the skin induces contact dermatitis, a commonly observed skin disease. Because repeated application of allergens induces chronic allergic lesions in the skin and a shift in the cutaneous cytokine milieu to Th2, this method is used to produce a mouse model of chronic ACD.^{13,14} In the present study, we prepared a Th2-dominant chronic ACD model, developed by repeated application of allergens, and investigated the role of Th17 cells and Th17-induced cytokines.

Methods

Animals and agents

Experiments were undertaken following the guidelines for the care and use of experimental animals of the Animal Research Committee of Sagami Women's University, which approved the study.

All experiments were performed using female C57BL/6 mice, 10–12 weeks old. Mice were housed with soft bedding, a 12-h light/dark cycle, and *ad libitum* access to food and water.

Sensitization and challenge procedure to induce chronic allergic contact dermatitis

The sensitization and challenge procedure is explained in Fig. 1a. TNCB was obtained from Sigma Chemical (St. Louis, MO, USA), dissolved in acetone to a 1% solution, and used for sensitization and challenge. Mice were sensitized by painting 40 µL of the 1% TNCB solution onto the lower dorsal skin (day 0). The 40 mice were then separated into five groups. Mice in group 1 (no-challenge control group) were not challenged, and skin and blood were collected on day 7. Mice in group 2 were challenged by painting 100 µL of 1% TNCB solution onto the lower dorsal skin on day 7 only. Mice in groups 3–5 were challenged every day using 20 µL of 1% TNCB solution on the sensitized lower dorsal skin, starting from day 7 after sensitization, and continuing until day 17 (group 3), day 27 (group 4) or day 37 (group 5). To investigate cytokine levels, biopsies were taken from the challenged lower back skin biopsies 24 h after the final challenge; that is, on day 8 for group 2 (1 challenge), day 18 for group 3 (11 challenges), day 28 for group 4 (21 challenges) and day 38 for group 5 (31 challenges). Blood

was also collected from each mouse at 24 h after the final challenge.

Injection procedure to investigate effects of interleukin-17 on T helper 2-dominant chronic allergic contact dermatitis

Mouse IL-17 [1 ng/g body weight in phosphate-buffered saline (PBS); R&D Systems, Minneapolis, MN, USA] was injected into the dorsal dermis of mice once daily during TNCB challenge. This method was performed to determine the effects of IL-17 on Th2-dominant chronic ACD by comparing the results with mice injected with PBS only.

Histological assessment

Frozen sections of skin biopsies (5 µm thick) were stained with haematoxylin and eosin. Epidermal thickness was measured under the microscope by a previously reported method.¹⁵ After immunohistochemical treatment with anti-IL-17F (1.0 µg/mL; Rockland, Gilbertsville, PA, USA), anti-IL-22 (2.0 µg/mL; GeneTex, Irvine, CA, USA) and anti-IL-23 antibodies (2.0 µg/mL; AnaSpec, San Jose, CA, USA), sections were stained using secondary antibodies. IL-17F+, IL-22+ and IL-23+ cells were quantitatively estimated in skin by counting the numbers of cells stained with anti-IL-17F, anti-IL-22 or anti-IL-23 antibody, respectively) in high-power fields.¹⁶

Measurement of cytokines

Skin samples were homogenized in PBS containing protease inhibitor (Complete™; Roche Diagnostics, Mannheim, Germany) for 2 min using a microtube homogenizer (Kenis, Osaka, Japan), and centrifuged at 5000 *g* for 5 min. Mouse ELISA kits for interferon (IFN)-γ, IL-4, IL-6, and IL-17 (all Ray Biotech, Norcross, GA, USA) and for IL-22, IL-23 and transforming growth factor (TGF)-β (all R&D Systems) were used to measure cytokine concentrations in the supernatants. Optical density at 450 nm for each well was measured using a microplate reader (Model 680; Bio-Rad Laboratories, Hercules, CA, USA).

Measurement of serum IgE

Concentrations of serum IgE were measured using a commercial kit (Mouse IgE ELISA kit; Shibayagi, Gunma, Japan) in accordance with the instructions from the manufacturer.

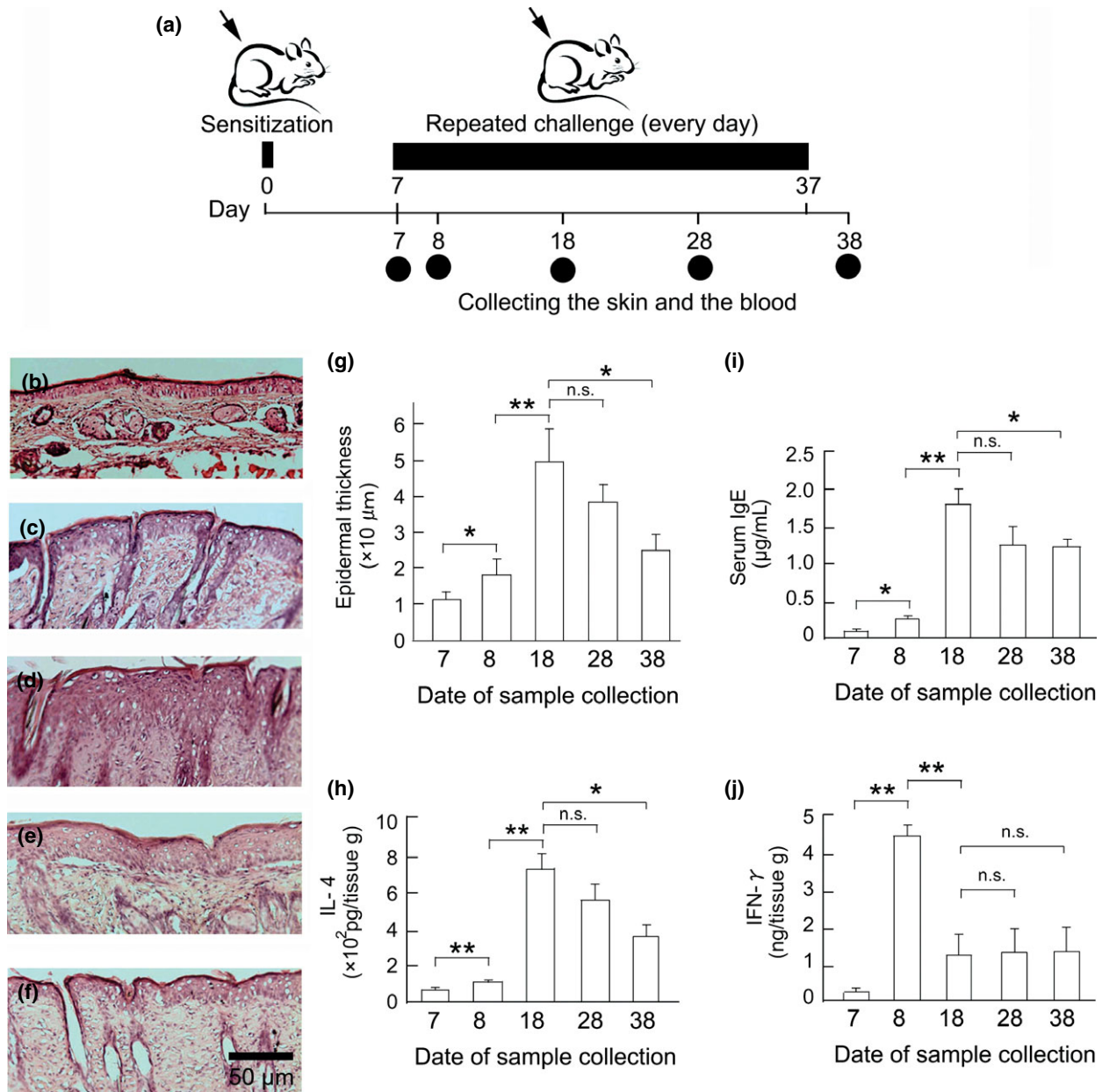


Figure 1 Histology and cytokine levels in allergic contact dermatitis (ACD). (a) Chronic ACD model. Application of 2,4,6-trinitro-1-chlorobenzene (TNCB) and sample collection are explained in the Methods section. (b–f) Sensitized and challenged skin lesions (haematoxylin and eosin, original magnification × 100): (b) day 7 (no challenge); (c) day 8 (challenge 1); (d) day 18 (challenge 11); (e) day 28 (challenge 21); and (f) day 38 (challenge 31). (g) Epidermal thickness, and levels of (h) interleukin (IL)-4, (i) serum IgE and (j) interferon (IFN)-γ. Data are expressed as mean ± SEM (*n* = 8). NS, not significant. **P* < 0.05, ***P* < 0.01.

Statistical analysis

Values are presented as mean ± SEM. Statistical significance between groups was analyzed using the two-tailed Student's *t*-test. Differences were considered statistically significant for values of *P* < 0.05.

Results

Chronic eczematous lesions on dorsal skin gradually improved after repeated challenge of 2,4,6-trinitro-1-chlorobenzene

At day 8 in group 2 (acute ACD), inflammatory cells were visible in the skin and there was mild oedema

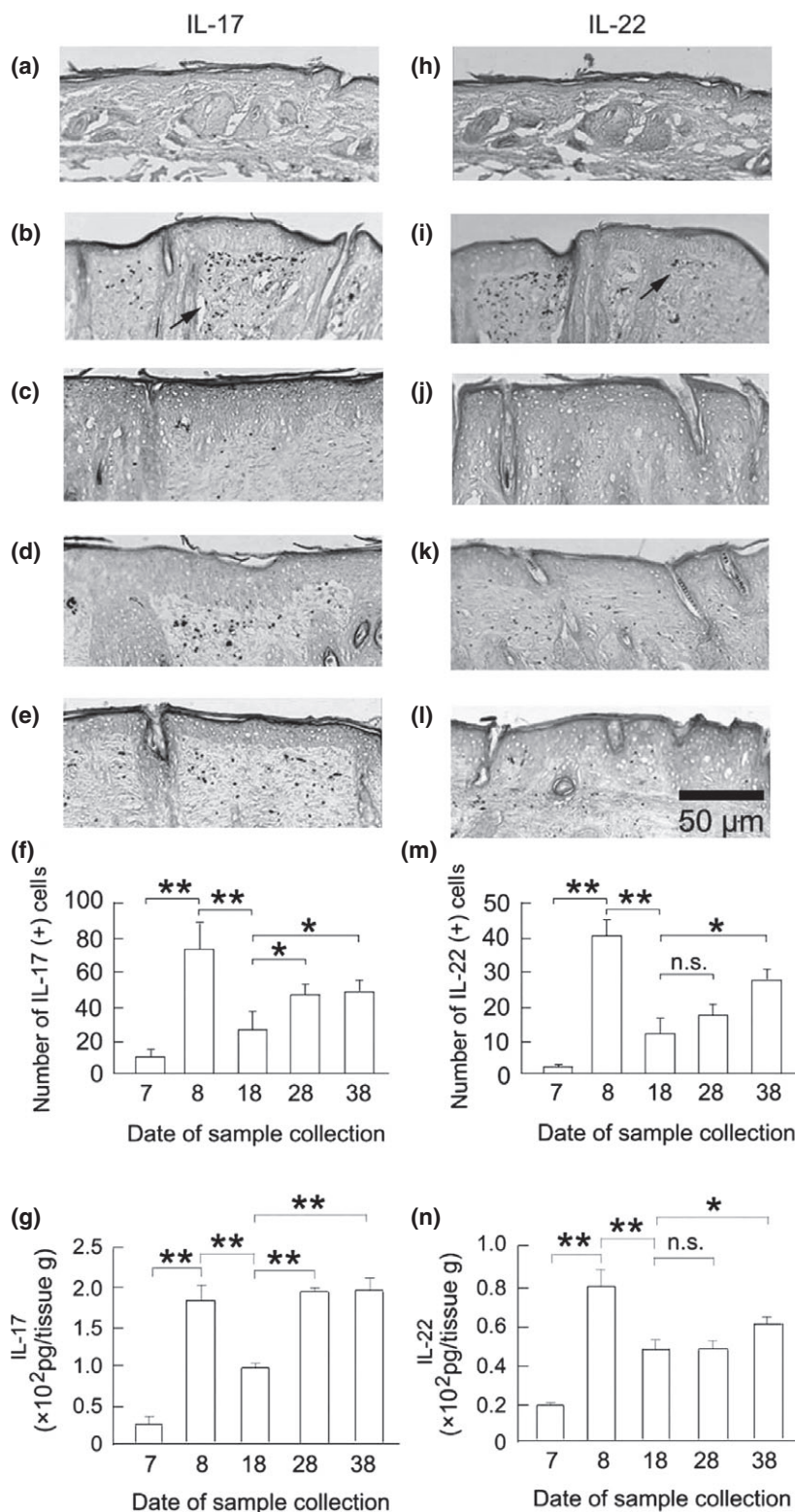


Figure 2 Interleukin (IL)-17 or IL-22 in allergic contact dermatitis (ACD). (a–e) IL-17F staining; (h–l) IL-22 staining. Original magnification (a–l) $\times 100$. (a,h) Day 7 (no challenge); (b,i) day 8 (challenge 1); (c,j) day 18 (challenge 11); (d,k) day 28 (challenge 21); and (e,l) day 38 (challenge 31), Arrow indicates IL-17F+ or IL-22+ cells. (f,m) Number of IL-17F+ and IL-22+ cells in challenged skin; (g,n) Levels of IL-17 and IL-22 in challenged skin. Data are expressed as mean $\pm$ SEM ($n = 8$). NS, not significant. * $P < 0.05$, ** $P < 0.01$.

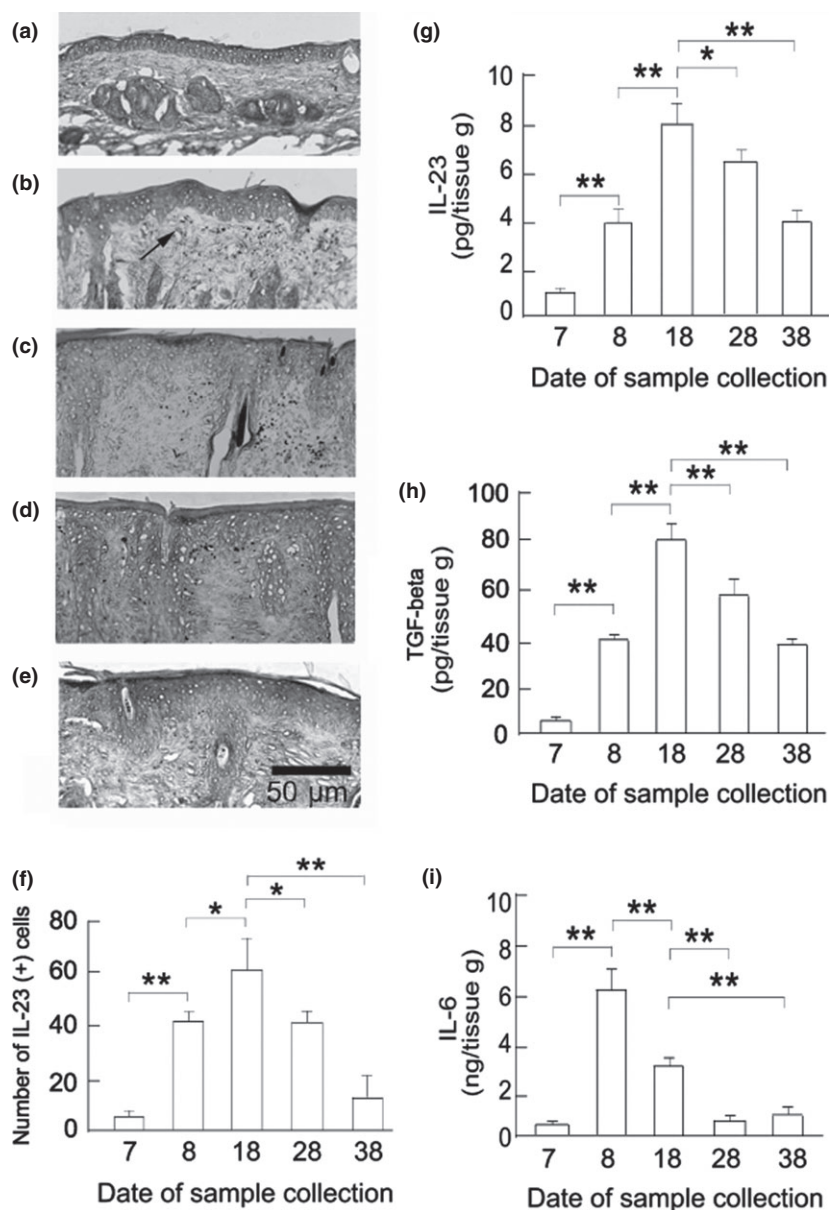


Figure 3 T helper (Th)17-induced cells and cytokine levels in allergic contact dermatitis (ACD). (a–e) Interleukin (IL)-23 staining. Original magnification $\times 100$. (a) Day 7 (no challenge), (b) day 8 (challenge 1); (c) day 18 (challenge 11); (d) day 28 (challenge 21); and (e) day 38 (challenge 31). Arrow indicates IL-23+ cell. (f) Number of IL-23+ cells unchallenged skin. Levels of (g) IL-23, (h) transforming growth factor (TGF)- β and (i) IL-6 in challenged skin. Data are expressed as mean $\pm$ SEM ($n = 8$). NS, not significant. * $P < 0.05$, ** $P < 0.01$.

(Fig. 1c). Marked epidermal hyperplasia with intercellular oedema and intense dermal cell infiltration was observed on day 18 in group 3 (Fig. 1d). Epidermal hyperplasia and inflammatory cell infiltration were ameliorated on days 28 and 38 for groups 4 and 5, respectively (Fig. 1e,f). Epidermal thickness was significantly increased on day 18 and gradually decreased thereafter (Fig. 1g). Following the development of severe scaly erythema with hair loss on day 18, skin lesions had improved by day 38.

Tissue interleukin-4 and serum IgE levels corresponded to the development and improvement of chronic eczematous lesions

Tissue levels of IL-4 in dorsal skin and serum levels of IgE on day 8 in group 2 were higher than in controls (group 1) (Fig. 1h,i). Marked increases were seen on day 18 in group 3, and decreases were apparent on days 28 and 38 in groups 4 and 5 (Fig. 1h,i). These trends showed a similar pattern to the extent of eczematous lesions. By contrast, tissue IFN- γ levels

increased dramatically on day 8, then decreased later (Fig. 1j).

T helper 17 cells infiltrated in a biphasic manner in the contact dermatitis model

We investigated levels of the Th17 cytokines IL-17 and IL-22, and found that IL-17F⁺ and IL-22⁺ cells were very scarce in control skin (Fig. 2a,f,h,m). These cells had infiltrated abundantly into the superficial dermis on day 8 (acute ACD) (Fig. 2b,f,i,m). However, the number of these cells decreased by day 18 (chronic ACD) (Fig. 2c,f,j,m), but they reappeared a little deeper in the dermis on days 28 and 38 (Fig. 2d–f,k–m). Tissue IL-17 and IL-22 levels showed similar trends, with elevations on day 8 and significant reductions on day 18. After that, IL-17 level rose again on days 28 and 38, whereas IL-22 level was increased by day 38 (Fig. 2g,n). Because IL-17 and IL-22 are known to colocalize in Th17 cells,¹ this profile can be explained simply by the biphasic emergence of Th17 cells.

Interleukin-23⁺ cells were abundant and tissue levels of T helper 17-induced cytokines increased in the early stage of chronic allergic contact dermatitis

We next tried to clarify levels of the cytokines known to trigger Th17 polarization. A few IL-23⁺ cells were seen in the unchallenged skin (Fig. 3a,f). IL-23⁺ cells emerged in the superficial dermis on day 8, then higher numbers were seen a little deeper in the dermis on day 18 (Fig. 3b,c,f), and from this point, the numbers gradually decreased (Fig. 3d–f). Levels of tissue IL-23 and TGF- β gradually increased, reaching their highest levels on day 18 and gradually decreasing thereafter (Fig. 3g,h). Tissue IL-6 level was highest on day 8, and decreased with repeated challenge (Fig. 3i).

T helper 2 response was inhibited by interleukin-17 in chronic allergic contact dermatitis

IL-17 was injected into the dorsal skin of mice once daily during TNCB challenge. The injected IL-17 significantly decreased tissue IL-4 levels on days 18 and 38 (Fig. 4a) and serum IgE levels on day 18 (Fig. 4b).

Discussion

Repeated challenge with allergens results in the development of chronic ACD,¹⁴ and alters the balance of locally released cytokines, with a shift towards Th2-dominated responses, reducing the more deleterious Th1 response in the skin.¹³ By contrast, acute ACD induced in a single challenge is Th1-dominant.¹³ In the current study, we found that the number of Th17 cells and the Th17 cytokine levels decreased in the early stage (day 18) of chronic ACD. This can be explained by the balance mechanism, as the cytokines required for Th1 and Th2 differentiation (IFN- γ , IL-12 and IL-4) inhibit Th17 differentiation.^{17, 18} Because the absence of both IL-17 and IFN- γ leads to further augmentation of Th2 differentiation in graft-versus host disease,¹² this mechanism explains the finding that decreases in IL-17 and IFN- γ levels on day 18 in chronic lesions follow the induction of the Th2-dominant response in our model. Th17 cytokines increased again on days 28 and 38 (late-stage chronic ACD) compared with those on day 18, whereas Th2 cytokines decreased.

In our results, IL-23 level was highest in the eczematous lesions on day 18. IL-23 activates the expression of Th17 cells¹ and amplifies the activity of Th17 through the IL-23 receptor.¹⁹ IL-23 acts on memory T cells, resulting in increased IL-17 and IL-17F secretion.²⁰ From these reports, we inferred that the increase of IL-23 in skin tissue induced Th17 differentiation and an increase in IL-17 level. When IL-23⁺ cells were at their

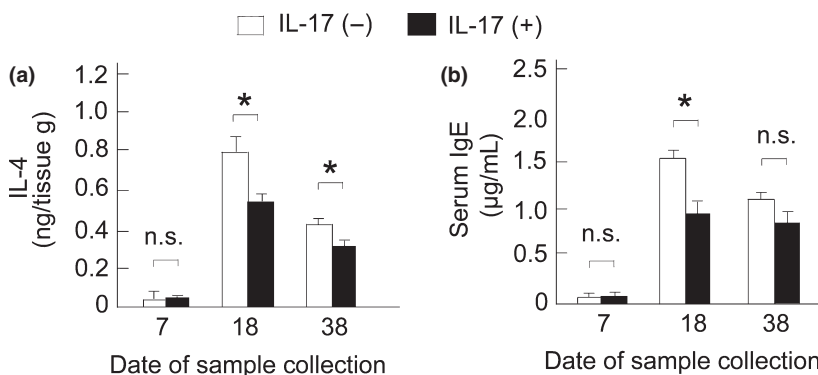


Figure 4 Interleukin (IL)-4 and IgE levels in IL-17 injected mice. (a,b) IL-17 was injected into the dorsal dermis of mice once daily during 2,4,6-trinitro-1-chlorobenzene (TNCB) challenge. (a) Tissue IL-4; (b) serum IgE. Data are expressed as mean $\pm$ SEM ($n = 6$). NS, not significant. * $P < 0.05$.

most abundant, they were located at a similar depth to the phase of the IL-17F⁺ and IL-22⁺ cell increment (Fig. 3c). We believe that these cells released IL-23 and were involved in the terminal differentiation of Th17 cells at that site. However, further study is needed to clarify these issues. TGF- β level in skin lesions was highest on day 18, similar to the IL-23 level. TGF- β is a cytokine critical for commitment to Th17 development, independent of IL-23.^{21,22} In the current study, we found that Th17 development on days 28 and 38 was under the influence of high levels of TGF- β . IL-6 level was relatively higher in the dorsal skin on day 18 than on days 28 and 38, even though the level was highest on day 8. IL-6 and TGF- β together induce the differentiation of pathogenic Th17 cells from naive T cells.²³ These observations suggest that increased levels of IL-23, TGF- β and IL-6 in combination on day 18 caused increased Th17 response, inducing decreases in Th2 response in the late stage of chronic ACD (days 28 and 38).

Increased levels of Th17 cytokines led to decreased Th2 response on days 28 and 38. This observation was further supported using IL-17 injection, which suppressed IL-4 and IgE levels; this effect was transmitted through IL-17.

Conclusion

Taken together, our results show that Th17 function is one of the important factors regulating chronic allergic diseases such as chronic contact dermatitis and atopic dermatitis, as these are Th2-dominant diseases. However, further studies to clarify the role of Th17 function in chronic allergic diseases are needed.

Acknowledgements

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What's already known about this topic?

- IL-17 is involved in contact hypersensitivity, in which Th1 response is greater than Th2 response.
- Chronic contact dermatitis induced by repeated elicitation of contact hypersensitivity changes into Th2-dominant response.

What does this study add?

- Chronic contact dermatitis is associated with increased Th2 response and decreased Th17 response.
- Th17 response is closely related to Th2 response in contact dermatitis.

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