

The role of interleukin-17 in mouse models of atopic dermatitis and contact dermatitis

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Summary

Background. Atopic dermatitis (AD) and contact dermatitis (CD) are both T cell-mediated eczematous disorders. Interleukin (IL)-17, expressed by T helper (Th)17 cells, is involved in recruitment of inflammatory cells into AD and CD skin.

Aim. In this study, we investigated whether IL-17 regulates immune dysregulation and affects skin barrier in oxazolone (OXA)-induced AD-like and CD-like disease models in mice, by comparing IL-17 null mutant (IL-17^{-/-}) vs. wild-type (WT) mouse strains in the models.

Methods. IL-17^{-/-} and WT Balb/c mice were used for OXA induction of AD-like and CD-like skin diseases. Ear swelling was measured by a micrometer. Skin biopsies were obtained for RNA isolation and histology. Real-time quantitative PCR analysis was performed to quantify mRNA expression of Th2 cytokines. Skin permeability was measured by a vapometer, and structural changes in the skin were evaluated by electron and confocal microscopy.

Results. Both OXA-induced AD and CD responses were alleviated in IL-17^{-/-} mice relative to WT, as demonstrated by reductions in ear swelling, inflammatory cell infiltration and levels of Th2 cytokines. These endpoints were used to characterize inflammatory dysregulation in both AD and CD models. Skin-barrier dysfunction, measured by increases in transepidermal water loss and dysfunction of lamellar bodies, and reductions in lipid distribution, were seen in both AD and CD in WT mice. In IL-17^{-/-} mice, however, these responses were significantly diminished.

Conclusions. The results indicate that the *IL-17* gene may play a role in modulating immune dysregulation and affecting skin barrier in OXA-induced AD-like and CD-like skin disease models in the Balb/c mouse.

Introduction

Atopic dermatitis (AD) is classified as a type I and IV allergic cutaneous hypersensitivity response, and contact dermatitis (CD) is classified as type IV.^{1,2} AD

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is a chronic inflammatory skin disorder that is characterized by a T helper (Th)2 cell-dominant inflammatory infiltrate and a defective epidermal permeability barrier against environmental agents.^{3,4} CD is a T-cell-mediated immune response that occurs as a result of chemical allergens (e.g. haptens) penetrating into the skin. There are clinical, histological, and immunological similarities between CD and acute AD.^{5,6}

Interleukin (IL)-17 is a proinflammatory cytokine produced by Th17 cells.⁷ There are six family members of IL-17, designated by the letters A-F. Levels of the IL-17 members IL-17A, IL-17E and IL-17F, and of

IL-17-producing cells, are known to be increased in the skin of patients with psoriasis or AD.^{7–11}

An important function of the SC is to act as a barrier protecting against invasion of foreign antigens, irritants and microorganisms.¹² A second function is regulation of transepidermal water loss (TEWL) through action of an intercellular lipid domain that is synthesized in and provided by the lamellar bodies (LBs) of keratinocytes.¹³ In general, a defective skin barrier facilitates penetration of allergens. In AD and CD, skin exhibits an impaired permeability barrier, which is associated with increased TEWL, diminished level of ceramides and abnormal epidermal proliferation and differentiation.^{12,14} Although a link between IL-17 and acute AD has been reported, the role of IL-17 in AD and CD is still unclear.

In this study, we investigated the role of IL-17 in immune response and skin barrier function in oxazolone (OXA)-induced AD-like and CD-like murine models.

Methods

Animals

All animal experiments in this study were performed in accordance with the Laboratory Animals Welfare Act and approved by the IACUC (Institutional Animal Care and Use Committee) of Yonsei University Health System (Seoul, Korea).

Female wild-type (WT) Balb/c mice, aged 6–8 weeks (Orient Bio; Sungnam, Korea) and IL-17 null mutant mice (IL-17^{−/−}) in a Balb/c background (gift from Y. K. Kim, POSTECH, Pohang, Korea) were used for the study. Generation of the IL-17^{−/−} mice was carried out as previously described.¹⁵ WT and IL-17^{−/−} mice were matched for age and sex. Mice were kept under controlled humidity, temperature and pathogen-free conditions.

Chemicals

OXA (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one; Sigma-Aldrich (St. Louis, MO, USA) was dissolved in a 4 : 1 mixture of acetone and olive oil.

Induction of atopic dermatitis

On day 0, IL-17^{−/−} and WT mice were sensitized with 5% (w/v) OXA or vehicle (the mixture of acetone and olive oil only) applied to the shaved dorsal skin. During days 5–23, the ears of the mice were also

challenged with 0.1% (w/v) OXA or vehicle, once every other day for 18 days. Ear swelling was measured with a micrometer (Mitutoyo; Kanagawa, Japan) and TEWL with a vapometer (Delfin; Kuopio, Finland) before every challenge and immediately before euthanasia, which occurred immediately after ear skin and blood samples were taken. On day 25, ear skin and blood samples were collected after mice were anaesthetized with zoletil (Virvac; Carros, France) and rompun (Bayer Korea; Ansan, Korea). OXA sensitization and challenge were performed by modifications of previously described protocols.¹⁶

Induction of contact dermatitis

On day 0, IL-17^{−/−} and WT mice were sensitized with 5% (w/v) OXA or vehicle applied to the dorsal skin. On day 5, the ears of the mice were challenged with 2% (w/v) OXA or vehicle. On day 6, TEWL and ear swelling were measured, then mice were anaesthetized, and ear skin and blood samples were collected. The mice were euthanized immediately afterwards.

TPA-induced contact dermatitis

Acute contact dermatitis was induced in ears of mice by a single application of 2 µg TPA (12-O-tetradecanoylphorbol-13-acetate; Sigma-Aldrich) dissolved in 20 µL of acetone to both the inner and outer ear surfaces. Ear swelling, TEWL and Th2 cytokine (IL-4 and IL-13) production were evaluated at 24 h after the application of TPA.

Histological analysis

Ears were fixed in 10% (w/v) formalin, embedded in paraffin wax, and cut into sections 4 µm thick, which were mounted onto slides, and stained with haematoxylin and eosin. Ear oedema and eosinophil recruitment were determined at ×100 and ×1000 magnification, respectively, using a light microscope (BX40; Olympus America, Center Valley, PA, USA).

Real-time quantitative polymerase chain reaction

Total RNA was isolated from ear skin using TRIzol (Invitrogen; Carlsbad, CA, USA) prior to complementary DNA (cDNA) synthesis. Synthesized cDNA was amplified (Exicycler™ 96, v3.0 (Bioneer; Daejeon, Korea) with a commercial mix (AccuPower® Dualstar™ qPCR PreMix; Bioneer) and primers (Bioneer). Primer details are shown in Table 1.

Table 1 Sequences of the primers used for PCR.

Gene	Direction	Sequence	Product size, bp
IL-17	Forward	TTAACTCCCTTGGCGCAAAA	21
	Reverse	CTTCCCTCCGCATTGACAC	20
IL-4	Forward	GGTCTCAACCCCGAGCTAGT	20
	Reverse	GCCGATGATCTCTCAAGTGAT	23
IL-13	Forward	CCTGGCTCTTGCTTGCTT	19
	Reverse	GGTCTTGCTGATGTTGCTCA	21
β -actin	Forward	GGCTGTATCCCTCCATCG	20
	Reverse	CCAGTTGGTAACAATGCCATGT	22

IL, interleukin.

Expression levels of target genes were quantified relative to the expression of β -actin, using the $2^{-\Delta\Delta C_t}$ method, as described in the manufacturer's protocol.

Electron microscopy

Ear skin specimens were pre-fixed in Karnovsky fixative (2% glutaraldehyde–paraformaldehyde in 0.1 mol/L phosphate buffer, pH 7.4) for 2 weeks. The specimens were rinsed in 0.1 mol/L phosphate buffer for 30 min, then post-fixed with 1% osmium tetroxide (OsO_4) dissolved in 0.1 mol/L phosphate buffer for 2 h, dehydrated in an ascending gradual series (50–100%) of ethanol, and infiltrated with propylene oxide. Specimens were embedded using a commercial kit (Poly/Bed 812 kit; Polysciences Inc., Warrington, PA, USA). Pure resin embedding and polymerization was carried out in a 60 °C electron microscopy oven (TD-700, DOSAKA, Tokyo, Japan) for 24 h. Sections 350 nm thick were cut and stained with toluidine blue for light microscopy. Sections 70 nm thick were cut on an ultramicrotome (Ultracut UCT; Leica Microsystems, Vienna, Austria), double-stained with 7% uranyl acetate and lead citrate for contrast staining, and observed under JEM-1011 transmission electron microscopy (JEOL, Tokyo, Japan) at an acceleration voltage of 80 kV. Images were obtained with a camera (MegaView III; Olympus, Tokyo, Japan).

Statistical analysis of data

SAS (v9.2; SAS Institute Inc.; Cary, NC, USA) and Graphpad Prism (v5; GraphPad Software, Inc.; La Jolla, CA, USA) were used, and all data are expressed as means $\pm$ SEM. Figures 1b and 3a were analysed using the linear mixed model in order to examine differences at a significant level of each group over

time. One-way and two-way ANOVA were performed for the other data, followed by Bonferroni *post hoc* tests. $P < 0.05$ was considered statistically significant.

Results

Reduction of ear oedema in IL-17^{-/-} mice with AD or CD

AD-like and CD-like skin lesions were induced by epicutaneous OXA exposure.^{3,16} Repeated applications of OXA to the ears induced an AD-like murine model (Fig. 1a). Strong ear swelling response was found in WT mice with AD, whereas IL-17^{-/-} mice had 50% less ear swelling after OXA exposure (Fig. 1b).

On histological analysis, reduction in ear oedema in IL-17^{-/-} mice with AD was clearly seen (Fig. 1c). Eosinophil recruitment was significantly increased in WT mice with AD, whereas IL-17^{-/-} mice with AD showed less eosinophil recruitment (Fig. 1d).

Unlike the AD protocol, a single application of OXA to the ears induced CD-like lesions in mice (Fig. 1e). Again, both reduction in ear oedema and eosinophil recruitment were observed in IL-17^{-/-} mice with CD, compared with WT mice with CD (Fig. 1f–h).

Reduction of T helper 2 cytokines in IL-17^{-/-} mice with atopic or contact dermatitis

In ears of WT mice with AD, significant induction of IL-17, IL-4 and IL-13 levels was seen. The levels were 33.5-fold, 17.5-fold and 6.7-fold higher, respectively, than levels in vehicle-treated WT mice. By contrast, expression levels of the Th2 cytokine and protein in IL-17^{-/-} mice with AD were significantly reduced. Expression of IL-17 was not detected in IL-17^{-/-} mice at all (Fig. 2a; Fig. S1a).

Reduction in Th2 cytokine and protein expression was also seen in IL-17^{-/-} mice with CD compared with WT mice with CD (Fig. 2b and Figure S1b).

Reduction of transepidermal water loss in IL-17^{-/-} mice with atopic or contact dermatitis

Water loss gradually increased over 20 days, from 6.4 to 29.3 g/m²/h in WT with AD, whereas TEWL reduced by about 0.5-fold in IL-17^{-/-} mice with AD (Fig. 3a). Reduction in TEWL was also higher in IL-17^{-/-} mice with CD than in WT mice with CD (Fig. 3b).

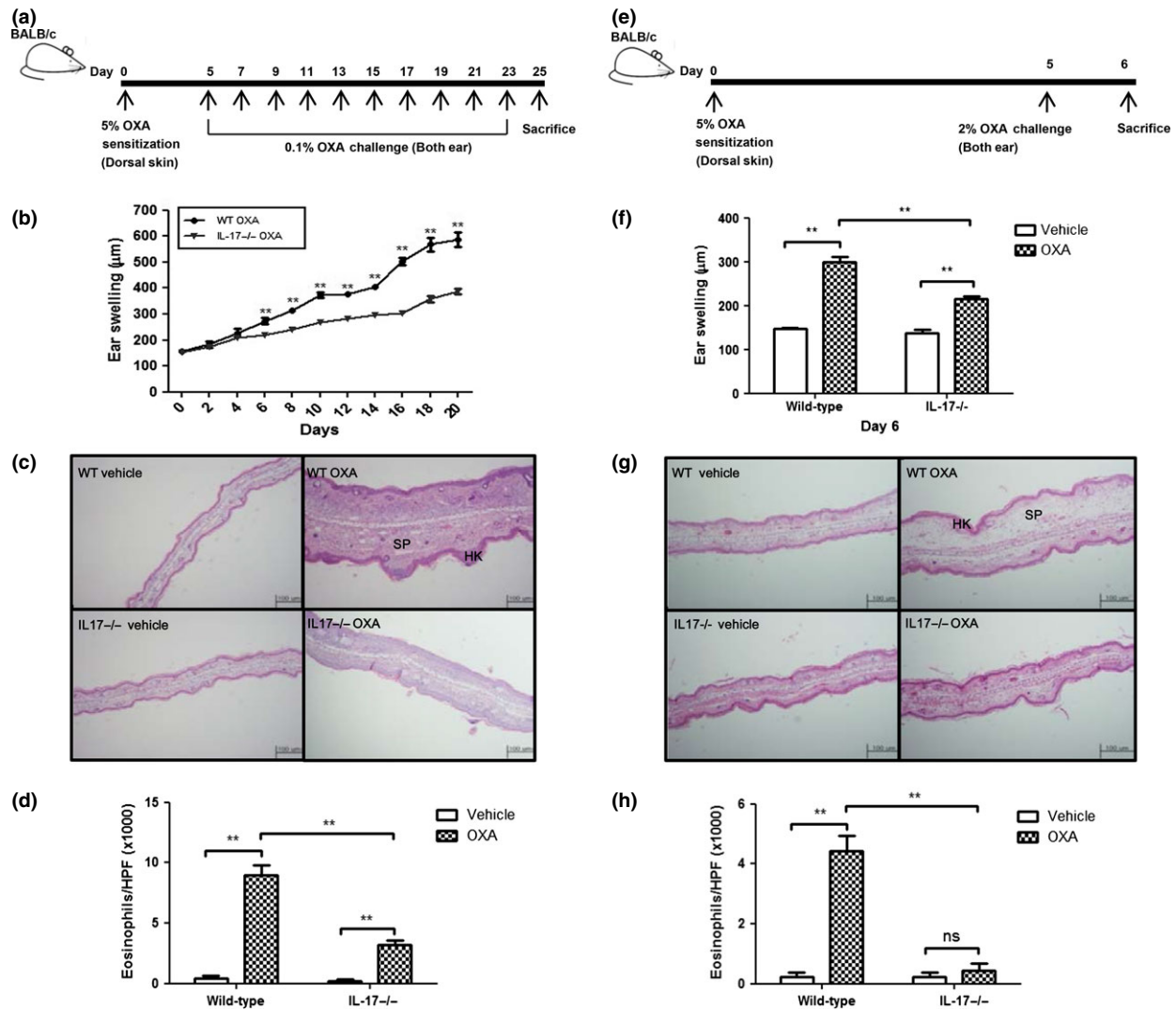


Figure 1 Changes in ear oedema and eosinophil recruitment after epicutaneous exposure to oxazolone (OXA). (a,e) Experimental protocol for (a) atopic dermatitis (AD) and (e) contact dermatitis (CD). Ear swelling response in (b) AD-like and (f) CD-like lesions. (c,g) Hyperkeratosis (HK) and thickening of oedema with spongiosis (SP) in (c) AD-like and (g) CD-like lesions. Haematoxylin and eosin, original magnification (c,d) $\times 100$. (d,h) The number of eosinophils was counted in high-power fields (HPF) in (d) AD-like and (h) CD-like lesions (haematoxylin and eosin, original magnification $\times 1000$), and expressed as cells per HPF. Three independent experiments ($n = 3-4$ mice/group). Data represent means $\pm$ SEM. $^{**}P < 0.001$ by (b) linear mixed model and (f,d,h) one-way or two-way ANOVA with Bonferroni *post hoc* tests.

Reduced dysfunction of lamellar bodies in IL-17^{-/-} mice with AD

An even distribution of LBs was seen in the stratum granulosum (SG) of vehicle-treated mice (Fig. 3c,d). It was observed that these single LBs moved toward the SC-SG junction, and the adjacent LBs extruded lipid into the SC-SG junction and formed lipid bilayers (Fig. 3g,h). However, the LB secretion system was impaired in WT mice with AD (Fig. 3e). As evidence for impaired LB, single LBs were found to be

agglomerated together, thus forming relatively large clusters (Fig. 3i). Reduced lipid bilayer (Fig. 3i) and increased LB densities (Fig. 3e) were observed at the SC-SG junction of AD compared with vehicle-treated WT mice. Both agglomeration of LB and disruption of the lipid bilayer (Fig. 3j) were reduced in IL-17^{-/-} mice with AD compared with WT mice with AD. LB counts were conducted because the agglomeration of LBs was not found in CD. The number of LBs was reduced at the SC-SG junction in CD lesions of WT

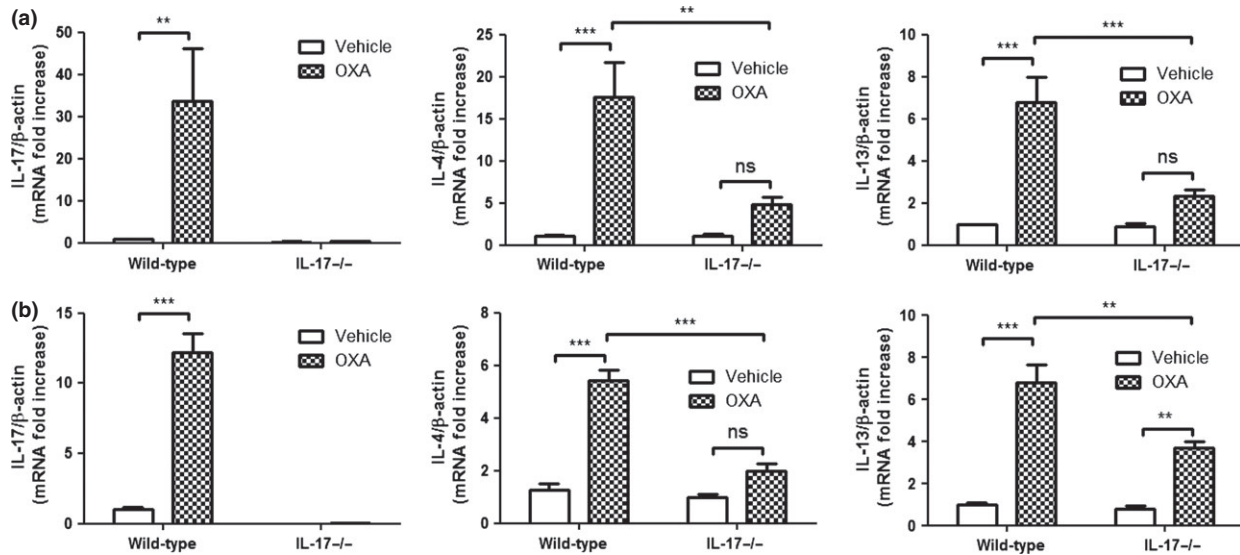


Figure 2 Expression of interleukin (IL)-17, IL-4 and IL-13 cytokines. (a,b) The level of cytokines was analysed by real-time PCR in (a) atopic dermatitis (AD)-like and (b) contact dermatitis (CD)-like lesions. Data represent means $\pm$ SEM. ** $P < 0.01$, *** $P < 0.001$ by one-way or two-way ANOVA with Bonferroni *post hoc* tests. Three independent experiments ($N = 3$ –4 mice/group).

mice. However, the number of LBs in IL-17^{-/-} mice with CD was rapidly restored (Fig. 3k), but there was no alteration in the number of LBs in the SG (Fig. 3l).

Discussion

It has been reported that AD and CD are caused by skin barrier dysfunction and immune dysregulation, and that IL-17 might play a pivotal role in allergic skin disease.^{9,10,14,17} Thus, to clarify the role of IL-17 in relation to the inflammatory response and skin barrier function in AD and CD, we established OXA-induced skin models of AD-like and CD-like lesions in WT and IL-17^{-/-} Balb/c mice. Using these strains, we found that IL-17^{-/-} mice showed lower AD-like and CD-associated responses than WT mice. The TPA-induced model, which is another type of dermatitis model, also showed similar responses (Figure S3). We then examined changes in skin barrier function, including TEWL, lipid distribution and LB clustering. Immune regulation was studied by ear swelling, eosinophil recruitment and Th2 cytokine expression in the presence vs. absence of IL-17.

We found ear oedema, eosinophil recruitment and expression of Th2 cytokines in the epidermis and dermis of OXA-induced AD-like or CD-like lesions in WT mice. In addition to decreased OXA-induced AD and CD responses in IL-17^{-/-} vs. WT, it was confirmed that the above inflammatory characteristics were relatively diminished in the skin of the IL-17-deficient strain. With respect to

the pathogenesis of AD, Cheung *et al.* reported that IL-17A and IL-17F are crucial for eosinophil production of IL-18, chemokine (C-X-C motif) ligand (CXCL)1 and chemokine (C-C motif) (CCL)4, as well as IL-1β and IL-6, and they proposed a possible interaction between Th17 cells and eosinophils in AD.^{8,18}

Hyperkeratosis and spongiosis, expressed in AD-like and CD-like lesions, are associated with mutation of keratin genes and damage to intercellular adhesions of keratinocytes, respectively. They contribute to an abnormal permeability barrier, which results in increased intracellular water movement.^{19,20} The epidermal barrier marker filaggrin has critical roles in the retention of water and formation of the skin barrier.¹² It was recently reported that filaggrin mutations in patients with AD raise the risk for contact sensitization.^{21,22} IL-17A diminishes the expression of filaggrin and other genes that could affect skin barrier formation in atopic eczema.²³ This suggests a possibility that IL-17 has direct effects on the skin barrier.

It was previously reported that dysfunction of LB secretion and delivery leads to global reduction of SC lipids in AD.^{3,4,6} Our data suggest that dysfunction of LB in IL17^{-/-} mice with AD is reduced, as we found lower levels of both LB agglomeration and disruption of LBL in these mice compared with WT mice with AD. Intracellular lipids assist in preventing internal water loss in forming a bilayer structure between corneocytes.⁴ Thus, increased TEWL is associated with disruption of SC lipids (Figure S2a,b). A recent study²⁴ demonstrated that

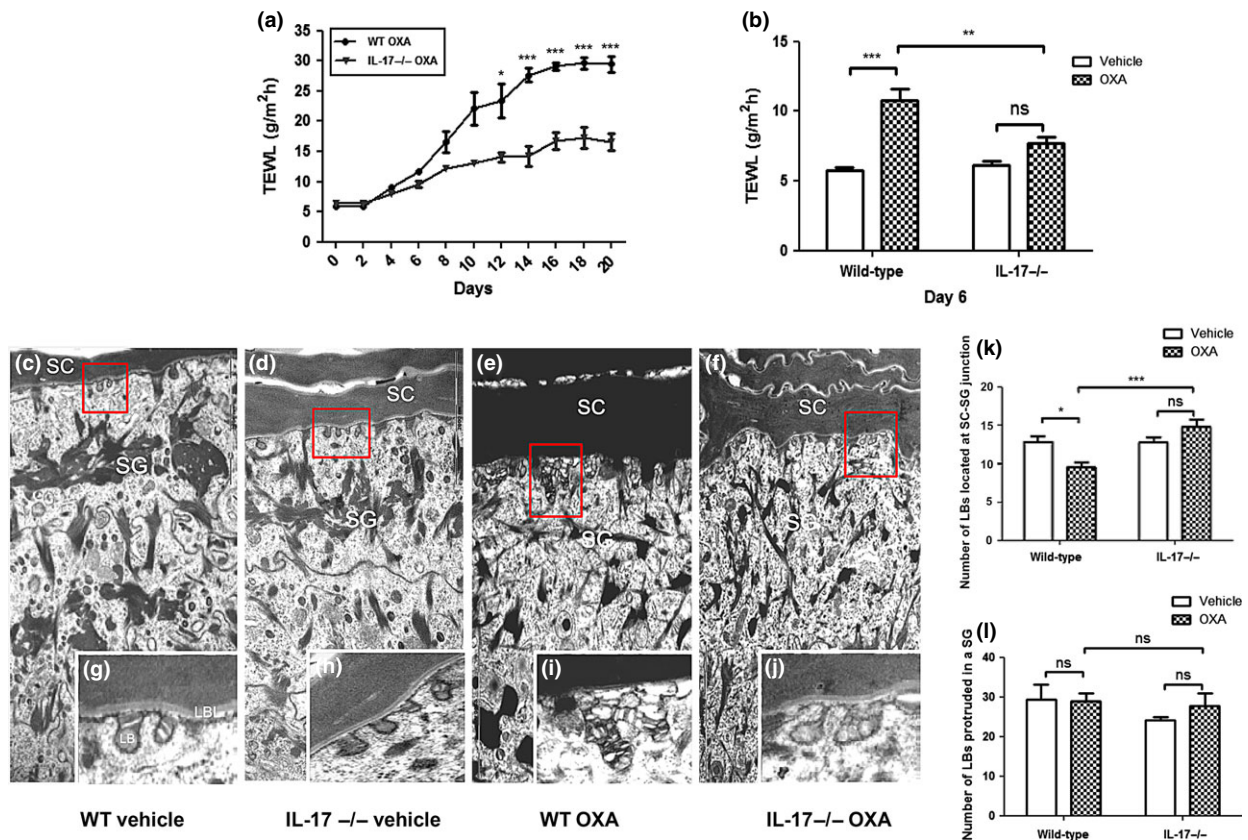


Figure 3 Changes in skin barrier. (a,b) Transepidermal water loss (TEWL) measurement in (a) atopic dermatitis (AD)-like and (b) contact dermatitis (CD)-like lesions. (c–j) Staining with osmium tetroxide (OsO₄) revealed the form (rectangle) and distribution of lamellar bodies (LB) at the stratum corneum–stratum granulosum (SC–SG) junction and in the SG in AD-like lesions. Original magnification (c–j) $\times 20\,000$; inset, $\times 50\,000$. (k,l) Distribution of LB seen by OsO₄ staining. The LBs were counted at the SC–SG junction and in the SG in CD-like lesions. Original magnification (k,l) $\times 20\,000$. Data represent means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by (a) linear mixed model and (b,k,l) one-way or two-way ANOVA with Bonferroni *post hoc* tests. Three independent experiments ($n = 4–5$ mice/group). Abbreviations: ns, not significant; LBL, lipid bilayers.

IL-17 regulates LB and lipid barrier formation genes such as sphingomyelin phosphodiesterase 1 (*SMPD1*) and serine palmitoyltransferase, long chain base subunit 2 (*SPTLC2*). Thus, these data show a correlation between IL-17 and LB system.

Acute permeability barrier disruption stimulates rapid secretion of preformed LBs in the uppermost SG cells, which quickly restores the skin barrier function. After this occurs, SG cells begin to rapidly synthesize new LBs.¹³ In OXA-induced CD-like lesions of WT mice, unlike AD lesions, LBs were not clustered, and the LB number was reduced at the SC–SG junction. Reduced LBs in CD-like lesions meant that the process of LB synthesis and transport from SG to SC was delayed after OXA challenge, resulting in reduced neutral lipid distribution. In the less damaged LBL of CD-like lesions in IL-17^{-/-} mice, there appeared to be rapid recovery of permeability barrier function.

Conclusion

In this study, it was demonstrated that IL-17 modulates Th2-type cytokine expression and affects skin barrier in OXA-induced AD-like and CD-like lesions in mice. Although further studies are needed to identify a correlation between IL-17 and the pathogenesis of skin hypersensitivity, our findings suggest that IL-17 might play a critical regulatory role in acute or chronic relapsing and inflammatory skin diseases.

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What's already known about this topic?

- Members of the IL-17 family are involved in Th2-mediated inflammatory responses such as AD.
- However, the role of IL-17 is still unclear in AD and CD.

What does this study add?

- IL-17 affects inflammatory reactions necessary for development of AD-like and CD-like disease responses.
- IL-17 may contribute to the damage of skin barrier in AD-like and CD-like models.
- Activation of IL-17 may affect LB form and system of secretion in an AD-like model.

References

- Mittermann I, Aichberger KJ, Bunder R *et al*. Autoimmunity and atopic dermatitis. *Curr Opin Allergy Clin Immunol* 2004; **4**: 367–71.
- Smith Pease CK, Basketter DA, Patlewicz GY. Contact allergy: the role of skin chemistry and metabolism. *Clin Exp Dermatol* 2003; **28**: 177–83.
- Jin H, He R, Oyoshi M, Geha RS. Animal models of atopic dermatitis. *J Invest Dermatol* 2009; **129**: 31–40.
- Cork MJ, Danby SG, Vasilopoulos Y *et al*. Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol* 2009; **129**: 1892–908.
- Akhavan A, Cohen SR. The relationship between atopic dermatitis and contact dermatitis. *Clin Dermatol* 2003; **21**: 158–62.
- Man MQ, Hatano Y, Lee SH *et al*. Characterization of a hapten-induced, murine model with multiple features of atopic dermatitis: structural, immunologic, and biochemical changes following single versus multiple oxazolone challenges. *J Invest Dermatol* 2008; **128**: 79–86.
- Koga C, Kabashima K, Shiraishi N *et al*. Possible pathogenic role of Th17 cells for atopic dermatitis. *J Invest Dermatol* 2008; **128**: 2625–30.
- Di Cesare A, Di Meglio P, Nestle FO. A role for Th17 cells in the immunopathogenesis of atopic dermatitis? *J Invest Dermatol* 2008; **128**: 2569–71.
- Pappu R, Ramirez-Carrozzi V, Ota N *et al*. The IL-17 family cytokines in immunity and disease. *J Clin Immunol* 2010; **30**: 185–95.
- van Beelen AJ, Teunissen MB, Kapsenberg ML *et al*. Interleukin-17 in inflammatory skin disorders. *Curr Opin Allergy Clin Immunol* 2007; **7**: 374–81.
- Toda M, Leung DY, Molet S *et al*. Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. *J Allergy Clin Immunol* 2003; **111**: 875–81.
- Proksch E, Folster-Holst R, Brautigam M *et al*. Role of the epidermal barrier in atopic dermatitis. *J Dtsch Dermatol Ges* 2009; **7**: 899–910.
- Feingold KR, Denda M. Regulation of permeability barrier homeostasis. *Clin Dermatol* 2012; **30**: 263–8.
- Proksch E, Brasch J. Abnormal epidermal barrier in the pathogenesis of contact dermatitis. *Clin Dermatol* 2012; **30**: 335–44.
- Nakae S, Komiyama Y, Nambu A *et al*. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 2002; **17**: 375–87.
- Park SY, Gupta D, Kim CH *et al*. Differential effects of peptidoglycan recognition proteins on experimental atopic and contact dermatitis mediated by Treg and Th17 cells. *PLoS One* 2011; **6**: e24961.
- Tokura Y, Mori T, Hino R. Psoriasis and other Th17-mediated skin diseases. *J UOEH* 2010; **32**: 317–28.
- Cheung PF, Wong CK, Lam CW. Molecular mechanisms of cytokine and chemokine release from eosinophils activated by IL-17A, IL-17F, and IL-23: implication for Th17 lymphocytes-mediated allergic inflammation. *J Immunol* 2008; **180**: 5625–35.
- Schmuth M, Yosipovitch G, Williams ML *et al*. Pathogenesis of the permeability barrier abnormality in epidermolytic hyperkeratosis. *J Invest Dermatol* 2001; **117**: 837–47.
- Li C, Lasse S, Lee P *et al*. Development of atopic dermatitis-like skin disease from the chronic loss of epidermal caspase-8. *Proc Natl Acad Sci USA* 2010; **107**: 22249–54.
- Hanifin JM. Filaggrin mutations and allergic contact sensitization. *J Invest Dermatol* 2008; **128**: 1362–4.
- Thyssen JP, Linneberg A, Ross-Hansen K *et al*. Filaggrin mutations are strongly associated with contact sensitization in individuals with dermatitis. *Contact Derm* 2013; **68**: 273–6.
- Gutowska-Owsiak D, Schaupp AL, Salimi M *et al*. IL-17 downregulates filaggrin and affects keratinocyte expression of genes associated with cellular adhesion. *Exp Dermatol* 2012; **21**: 104–10.
- Chiricozzi A, Nograles KE, Johnson-Huang LM *et al*. IL-17 induces an expanded range of downstream genes in reconstituted human epidermis model. *PLoS One* 2014; **9**: e90284.