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IL-17 Receptor A Maintains and Protects the Skin Barrier To Prevent Allergic Skin Inflammation

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Atopic dermatitis (AD) is a common inflammatory skin disease affecting up to 20% of children and 3% of adults worldwide and is associated with dysregulation of the skin barrier. Although type 2 responses are implicated in AD, emerging evidence indicates a potential role for the IL-17A signaling axis in AD pathogenesis. In this study we show that in the filaggrin mutant mouse model of spontaneous AD, IL-17RA deficiency (*Il17ra*^{-/-}) resulted in severe exacerbation of skin inflammation. Interestingly, *Il17ra*^{-/-} mice without the filaggrin mutation also developed spontaneous progressive skin inflammation with eosinophilia, as well as increased levels of thymic stromal lymphopoietin (TSLP) and IL-5 in the skin. *Il17ra*^{-/-} mice have a defective skin barrier with altered filaggrin expression. The barrier dysregulation and spontaneous skin inflammation in *Il17ra*^{-/-} mice was dependent on TSLP, but not the other alarmins IL-25 and IL-33. The associated skin inflammation was mediated by IL-5-expressing pathogenic effector Th2 cells and was independent of TCRγδ T cells and IL-22. An absence of IL-17RA in nonhematopoietic cells, but not in the hematopoietic cells, was required for the development of spontaneous skin inflammation. Skin microbiome dysbiosis developed in the absence of IL-17RA, with antibiotic intervention resulting in significant amelioration of skin inflammation and reductions in skin-infiltrating pathogenic effector Th2 cells and TSLP. This study describes a previously unappreciated protective role for IL-17RA signaling in regulation of the skin barrier and maintenance of skin immune homeostasis. *The Journal of Immunology*, 2017, 199: 707–717.

Atopic dermatitis (AD) is frequently recognized as the first manifestation of allergic disease in early life, with 15–30% of children affected in the developed world (1). The development of AD is dependent on interplay between genetic and environmental factors involving a disruption in skin homeostasis altering the functional integrity of the skin and leading to aberrant inflammation. In AD patients, inflammation in the acute lesions is associated with infiltration of IL-4-, IL-5-, and IL-13-expressing CD4⁺ Th 2 cells, eosinophils (2), and type 2 innate lymphoid cells (ILCs). Although this is indicative of a type 2 atopic inflammation, there is a recognition that other cytokines, including IL-17A and IL-22, may also contribute to the pathogenesis of AD (2).

Dysregulation of the IL-17A/IL-17RA axis is implicated in inflammatory skin diseases, with Abs against IL-17A and IL-17RA showing significant efficacy for the treatment of psoriasis (3). In AD patients, the increased expression of IL-17A in skin lesions raises the potential to target IL-17RA and its ligands IL-17A and IL-17F as a possible therapeutic strategy (4, 5).

In a subgroup of AD patients, defects in the integrity of the skin barrier arise due to mutations in the filaggrin (*FLG*)-encoding gene, with the filaggrin protein having a central role in normal skin barrier function and barrier integrity maintenance (6). The flaky tail mouse strain, which has natural mutations in *Flg* and also *Tmem79*, develops AD-like skin inflammation analogous to the inflammation that occurs in AD patients with *FLG* mutations (7–9). Recently, we generated a mouse strain with only the *Flg* mutation that has a compromised skin barrier and develops spontaneous AD-like inflammation when maintained on the Th2/17-prone BALB/c strain background, with skin inflammation characterized by elevated Th2 cytokines, as well as elevated IL-17A (10). In this study, it was noted that when *Flg* mutant mice were crossed to IL-17RA-deficient mice, they developed severely exacerbated allergic skin inflammation. Unexpectedly, *Il17ra*^{-/-} mice, in the absence of the *Flg* mutation, also developed spontaneous and progressive skin inflammation. IL-17RA-deficient mice had aberrant filaggrin expression in the skin, a defective skin barrier, and cutaneous inflammation associated with skin eosinophilia and elevated IL-5 and thymic stromal lymphopoietin (TSLP), with no contribution from IL-22 or TCRγδ⁺ T cells. The development of allergic skin inflammation due to IL-17RA deficiency was dependent on CD4⁺ T cells with a significant role for IL-5-producing pathogenic effector Th2 (peTh2) cells. Interestingly, absence of IL-17RA in the nonhematopoietic, but not in the hematopoietic compartment, was required for the induction of skin inflammation. Deficiency in TSLP-mediated signals, but not IL-25 or IL-33, restored barrier homeostasis and protected from the development of spontaneous allergic skin inflammation. IL-17RA deficiency also resulted in

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Abbreviations used in this article: AD, atopic dermatitis; FLG, filaggrin; ILC, innate lymphoid cell; peTh2, pathogenic effector Th2; TEWL, transepidermal water loss; TSLP, thymic stromal lymphopoietin; WT, wild-type.

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skin microbiome dysbiosis, whereas antibiotic treatment ameliorated skin inflammation and led to a significant reduction in the frequency of pTh2 cells. These results uncover a novel role of IL-17RA signaling in maintaining barrier integrity and immune system homeostasis in the skin.

Materials and Methods

Mice

All mice were on a congenic BALB/c strain with BALB/c mice used as wild-type (WT) controls. *Il17ra*^{-/-} mice on a C57BL/6 background were provided by Amgen (Thousand Oaks, CA) and backcrossed in-house to BALB/c mice. *Rag1*-deficient (*Rag1*^{-/-}), *Il22*-deficient (*Il22*^{-/-}), *TCRδ*-deficient (*TCRδ*^{-/-}) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). *Il5*-cerulean fluorescent protein (*Il5*^{cpfp}) (10), filaggrin mutant (*Flg*^{fl/fl}) (10), IL-17BR-deficient (*Il17br*^{-/-}), IL-33-deficient (*Il33*^{cit/cit}) (11), IL-33 receptor-deficient (*Il1rl1*^{-/-}), filaggrin-deficient (*Flg*^{-/-}) (12), and mice deficient in *Il4*, *Il5*, *Il9*, and *Il13* (*Il4/5/9/13*^{-/-}) (13) were described previously. *Il17ra*^{-/-} mice were crossed to *Flg*^{fl/fl}, *Rag1*^{-/-}, *Tslpr*^{-/-}, *Il17br*^{-/-}, *Il1rl1*^{-/-}, *Il33*^{cit/cit}, *Il17br*^{-/-}, *Il22*^{-/-}, *TCRδ*^{-/-} mice to generate dual-deficient mice. Mice were housed in specific pathogen-free conditions, with irradiated diet, bedding, and water ad libitum. All animal experiments were performed in compliance with the Irish Department of Health and Children and approved by the Trinity College Dublin's BioResources Ethical Review Board.

Clinical scoring of skin

Clinical skin severity of inflammation was scored blindly using macroscopic criteria as previously described (10). Briefly, scores of 0, none; 1, mild; 2, moderate; and 3, severe were applied to the signs of edema, erythema, scaling, and erosion. The total score for each mouse, maximum score of 12, was calculated from the sum of individual scores for each parameter.

Histology

Skin tissue samples were placed in 4% neutral buffered formalin. Following fixation, samples were processed in paraffin wax, sectioned, and stained by H&E. Epidermal thickness and cell infiltration were analyzed as described (10). Images were acquired using a Leica Microsystems DM3000 LED microscope with an attached DFC495 camera; image analysis was performed on LAS v4.0 software (Leica Microsystems).

MC903 treatment

MC903 (calcipotriol) was dissolved in ethanol; mice were treated daily for 4 consecutive days as previously described (14). Briefly, 25 μ l with a total of 4 nmol of MC903 were applied on one ear; the second ear was used as an internal control and was treated with ethanol only. Mice were scored daily and were sacrificed 24 h after the final treatment with MC903.

ELISA

The detection of cytokines (IL-4, IL-5, IL-13, IL-17A, IL-22, IL-1 β , IL-25, IL-33, and TSLP) from culture supernatant or tissue lysates was performed via ELISA according to the manufacturer's (R&D Systems) instructions.

RNA isolation and real-time PCR

RNA was isolated from skin of 5 wk noninflamed and 12 wk inflamed *Il17ra*^{-/-} mice and sex- and age-matched controls. RNA isolation was performed by using the RNeasy kit (Qiagen) and was followed by reverse transcription with the Quantitect reverse transcription kit incorporating a genomic DNA elimination step (Qiagen) as per the manufacturer's instructions. Real-time quantitative PCR was performed on an AB StepOnePlus real-time PCR system (Life Technologies), using predesigned TaqMan gene expression assays specific for murine *Tslp* (Mm00498739_m1), *Il25* (Mm00499822_m1), *Il33* (Mm01195784_m1), *S100a7* (Mm01218201_m1), *S100a8* (Mm00496696_m1), *S100a9* (Mm00656925_m1), *Defb2* (Mm00657074_m1), and *Cramp* (Mm00438285_m1). Specific gene expression was normalized to murine GAPDH (VIC probe, 4352339E; Life Technologies). Fold expression was calculated by using the comparative cycle threshold method of analysis and is presented as relative quantification.

Transepidermal water loss

The measurement of transepidermal water loss (TEWL) was performed on ear skin of mice using a Courage and Khazaka Tewameter TM210 (EnviroDerm, Evesham, U.K.), as described previously (7). TEWL was recorded at ambient temperature 19–21°C and humidity 50 $\pm$ 5%.

Western blotting

Western blot analysis of skin for filaggrin detection was performed as previously described (10). In brief, epidermis was separated from dermis following incubation in PBS with 5 mM EDTA at 56°C. Epidermis was homogenized in protein extraction buffer (50 mM Tris, 8 M urea [pH 7.6] with HALT protease inhibitor mixture; Thermo Scientific) using an Ultra-Turrax. Lysates were clarified by centrifugation (13,000 $\times$ g, 4°C for 15 min). Proteins were resolved by SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (Thermo Scientific). Membrane-bound proteins were incubated with primary rabbit anti-mouse filaggrin Ab (Covance) and secondary HRP-conjugated goat anti-rabbit IgG (Dako). ECL substrate (Thermo Scientific) was used to visualize membrane-bound filaggrin.

Primary keratinocyte culture

Culture of primary murine keratinocytes was performed as previously described (15). Briefly, the epidermis was separated from the dermis of neonatal mouse skin after overnight incubation at 4°C in 0.25% trypsin (Lonza). Cells were resuspended in KGM-2 BulletKit media (Lonza), adjusted to 0.05 mmol/l Ca²⁺, supplemented with penicillin-streptomycin (50 U/ml, 50 μ g/ml; Life Technologies). Cells were plated on fibronectin/collagen-coated six-well plates and cultured at 36°C at 5% CO₂ for 4 d, or to 90% confluence. Keratinocytes were differentiated in KGM-2 BulletKit media adjusted to 0.5 mmol/l Ca²⁺. Cells were harvested for analysis at 72 h following the initiation of differentiation and were analyzed by Western blot for filaggrin expression.

Cell transfer

CD4⁺ T cells were isolated from BALB/c mice or from the indicated donor mice by negative selection using magnetic separation on an autoMACS Pro cell separator (Miltenyi Biotec) according to the manufacturer's instructions. A total of 1 $\times$ 10⁷ CD4⁺ cells, >92% purity, were transferred i.v. to *Il17ra*^{-/-}*Rag1*^{-/-} recipient mice.

Flow cytometry

Single-cell suspension was prepared and Fc receptors were blocked with anti-CD16/anti-CD32 (24G2; eBioscience) in a 2% FBS in PBS solution. Cells were incubated with combinations of the following anti-mouse Abs against CD3 (145-2C11), CD4 (GK1.5), CD45.2 (104), CD193 (J073E5) (BioLegend), and Siglec-F (E50-2440; BD Biosciences). Samples were stained with a fixable Live/Dead stain (Aqua; Thermo Scientific) or propidium iodide (Sigma-Aldrich). For intracellular staining, cells were fixed and permeabilized using a commercial intracellular staining Cytofix/Cytoperm kit (BD Biosciences) according to the manufacturer's instructions. Cells were stained with anti-mouse cytokine Abs for IL-4 (11B11), IL-13 (eBio13A), (eBioscience), and IL-5 (TRFK5) (BD Biosciences). All samples were acquired using a Beckman Coulter CyAn ADP using Summit (v4.3) whereas analysis was performed with FlowJo (v8.87).

Cell stimulation and intracellular cytokine staining

All cell cultures were performed in the presence of RPMI 1640 (Invitrogen) supplemented with 10% heat-inactivated FBS (Sigma-Aldrich), 2 mmol L-glutamine (Invitrogen), 50 IU/ml penicillin, and 50 μ g/ml streptomycin (Invitrogen). Cells were stimulated in vitro with plate-bound anti-CD3 (10 μ g/ml, 2C11; BD Biosciences) and anti-CD28 mAb (2 μ g/ml, 37.51; BD Biosciences) for 72 h, followed by 50 ng/ml PMA and 1 μ g/ml ionomycin for 1 h prior to a 4-h incubation with 2 μ M monensin (eBioscience)-supplemented media. Cell culture supernatants were collected and cells were stained for flow cytometry analysis as described above.

Bone marrow chimeras

Bone marrow chimeric mice were generated as previously described (16). In brief, donor bone marrow cells were prepared from *Il17ra*^{-/-} or WT mice. Bone marrow cells (2 $\times$ 10⁷) were reconstituted in 100 μ l of PBS and i.v. injected into sublethally irradiated (900 rad in two doses of 500 and 400 rad) WT or *Il17ra*^{-/-} mice. Bone marrow chimeric mice were scored weekly for skin inflammation and weight changes.

Skin microbiome analysis

Skin microbiome samples were collected by directly exposing sterile swabs to ear skin of *Il17ra*^{-/-} and sex- and age-matched control mice. The same surface area was sampled for all mice, and to avoid sample cross-contamination fresh sterile gloves were used for each collection. Samples were instantly frozen in liquid nitrogen, and 16S rRNA gene sequencing and microbiome community analysis was performed by Second Genome (San Francisco, CA), as described previously (17).

Antibiotic treatment and cohousing studies

Mice were treated with a broad-spectrum antibiotic treatment protocol, as described previously (18). Briefly, 12-wk-old female WT and *Il17ra*^{-/-} mice were treated daily by oral gavage for 10 consecutive days with vancomycin (0.5 mg/ml), neomycin (1 mg/ml), metronidazole (1 mg/ml), ampicillin (1 mg/ml), and gentamicin (1 mg/ml) (Sigma-Aldrich) prepared in 0.2 ml of autoclaved water. Control mice received 0.2 ml of autoclaved water daily by oral gavage. Microbiome transfer was achieved by cohousing WT mice that had been treated for 10 d with the antibiotic regimen, as above, with untreated *Il17ra*^{-/-} mice as microbiome donors for 8 wk. As a control group, WT mice treated with antibiotics were cohoused with untreated WT mice as microbiome donor mice. Mice were scored weekly for skin inflammation, as above.

Statistical analyses

Data are expressed as mean $\pm$ SEM and were analyzed by two-way ANOVA test or unpaired Student *t* tests (Prism 6; GraphPad Software). A *p* value <0.05 was considered significant (**p* <0.05 , ***p* <0.01 , ****p* <0.001).

Results

IL-17RA deficiency leads to spontaneous progressive allergic skin inflammation

We have previously described that filaggrin mutant mice (*Flg*^{fl/fl}) have a defective skin barrier and are predisposed to developing AD-like skin inflammation that was associated with elevated levels of IL-17A (10). To investigate the role of IL-17A in spontaneous generation of AD-like inflammation in *Flg* mutant mice, dual *Il17ra*^{-/-} and *Flg*^{fl/fl} (*Il17ra*^{-/-}*Flg*^{fl/fl}) mice were generated on a BALB/c background. Unexpectedly, rather than ameliorating disease, in the absence of IL-17RA signaling adult filaggrin mutant mice developed exacerbated skin inflammation (Fig. 1A). Strikingly, the IL-17RA-deficient mice developed spontaneous skin inflammation, irrespective of the presence of the *Flg* mutation (Fig. 1A). The spontaneous skin inflammation in *Il17ra*^{-/-} mice developed after 6 wk of age and was progressive (Fig. 1B) and associated with high levels of serum IgE (Fig. 1C). *Il17ra*^{-/-} mice have a defective skin barrier that predates the development of overt skin inflammation, with significantly (*p* <0.05) elevated TEWL in 5-wk-old IL-17RA-deficient mice that have no overt skin inflammation (Fig. 1B), relative to age-matched WT mice (Fig. 1D). After the onset of skin inflammation in *Il17ra*^{-/-} mice there was pronounced barrier disruption, with significantly (*p* <0.001) increased TEWL in older (10-wk-old) mice (Fig. 1D). Our initial interest in IL-17RA arose from studies on filaggrin mutant mice, which also have a defective skin barrier (10). The filaggrin protein has a pivotal role in maintaining skin barrier integrity, is synthesized as a precursor protein (profilaggrin) in keratohyalin granules within the stratum corneum, and is subsequently proteolytically processed into filaggrin monomers (19). Alterations in filaggrin processing, including loss of filaggrin expression and altered premature filaggrin processing, have been linked with impaired skin barrier function (20, 21). A previous study has shown that IL-17A treatment of human keratinocytes directly altered filaggrin production and processing, leading to reduced expression of filaggrin monomers (22). Using in vitro-differentiated primary murine keratinocytes from WT mice, IL-17A treatment also reduced filaggrin monomer formation (Supplemental Fig. 1A). Similarly, primary keratinocytes from *Il17ra*^{-/-} mice constitutively express increased levels of filaggrin monomer when compared with keratinocytes from WT mice (Supplemental Fig. 1B). Importantly, Western blot analysis of epidermal skin from 5-wk-old *Il17ra*^{-/-} mice, with barrier disruption but no frank skin inflammation (Fig. 1), recapitulates the results from the in vitro-differentiated primary keratinocytes, with increased filaggrin monomer expression in the epidermis of *Il17ra*^{-/-} mice when compared with filaggrin expression in the epidermis of age-matched WT mice (Supplemental

Fig. 1C), and increased filaggrin expression in epidermis of skin sections of IL-17RA-deficient mice (Supplemental Fig. 1D). These data demonstrate aberrant filaggrin processing in the absence of IL-17RA-mediated signaling, which can have detrimental effects on the homeostasis of the skin barrier.

The IL-17RA-deficient mice used in this study were originally generated on a C57BL/6 background and backcrossed congenic to the BALB/c background in-house. C57BL/6 *Il17ra*^{-/-} mice do not develop spontaneous skin inflammation (Supplemental Fig. 2A, 2B) or elevated IgE (Supplemental Fig. 2C). It is relevant that these C57BL/6 *Il17ra*^{-/-} mice were bred and housed in the same animal facility, thus with identical environmental conditions, as the BALB/c IL-17RA-deficient mice that do develop skin inflammation (Fig. 1). However, in the MC903-induced model of AD-like skin inflammation C57BL/6 IL-17RA-deficient mice had significantly (*p* <0.001) accelerated skin inflammation when compared with C57BL/6 controls (Supplemental Fig. 2D), with a significant (*p* <0.05) increase in Th2 (GATA3⁺CD4⁺) cells (Supplemental Fig. 2E). Therefore, although *Il17ra*^{-/-} mice on a C57BL/6 strain background do not spontaneously develop AD-like inflammation, they do have a lower threshold to skin inflammatory insult compared with WT C57BL/6 strain mice.

The spontaneous AD-like skin inflammation and elevated IgE in *Il17ra*^{-/-} mice indicates potential roles for the cardinal type 2-associated cytokines (IL-4, IL-5, and IL-13). We therefore examined the expression of these cytokines in nonlesional skin. In the skin of 5-wk-old *Il17ra*^{-/-} mice, prior to the development of overt clinical skin inflammation, there were significantly (*p* <0.05) increased levels of IL-5 protein but decreased levels of both IL-4 (*p* <0.001) and IL-13 (*p* <0.05 ; Fig. 1E). Upstream of these Th2 cytokines, the alarmin cytokines IL-25, IL-33, and TSLP are key initiators of type 2-associated inflammation in epithelial tissues, including the skin (23, 24). Prior to the appearance of overt skin inflammation in the skin of 5-wk-old *Il17ra*^{-/-} mice, TSLP, but not IL-33 or IL-25, expression was significantly (*p* <0.001) elevated (Fig. 1F). Furthermore, the mRNA levels of TSLP, but not IL-25 or IL-33, were >30 -fold increased when compared with WT mice at 5 (no inflammation) and 12 wk of age, when clinical signs of inflammation were evident (Supplemental Fig. 3A). As with 5-wk-old noninflamed *Il17ra*^{-/-} mice, 12-wk-old inflamed *Il17ra*^{-/-} mice have significantly reduced levels of IL-4 (*p* <0.05) and IL-13 (*p* <0.05), but no alteration in IL-5, in the skin when compared with WT controls (Supplemental Fig. 3B). These results are consistent with a previous study showing reduced skin IL-4 expression in a mouse model of AD-like skin inflammation in the absence of IL-17A-mediated signals (25). It is noteworthy that in the skin of *Il17ra*^{-/-} mice protein levels of IL-17A were elevated, as reported previously (26, 27), whereas other cytokines, including IL-22 and IL-1 β , were not significantly different when compared with controls (Supplemental Fig. 3C). There was significant (*p* <0.0001) infiltration of T cells and, consistent with elevated expression of IL-5, eosinophils (*p* <0.01) in the skin of *Il17ra*^{-/-} mice when compared with control mice (Fig. 1G). In contrast, ILC2 and basophil populations, both implicated in AD, were not expanded in the skin of *Il17ra*^{-/-} mice (data not shown). These results highlight potential roles for IL-5 and TSLP in the early genesis of spontaneous allergic skin inflammation in IL-17RA-deficient mice.

Nonhematopoietic IL-17RA expression protects from spontaneous AD-like skin inflammation

To assess whether IL-17RA deficiency in the hematopoietic or the nonhematopoietic cell compartment is required for the development of spontaneous skin inflammation, we generated bone marrow

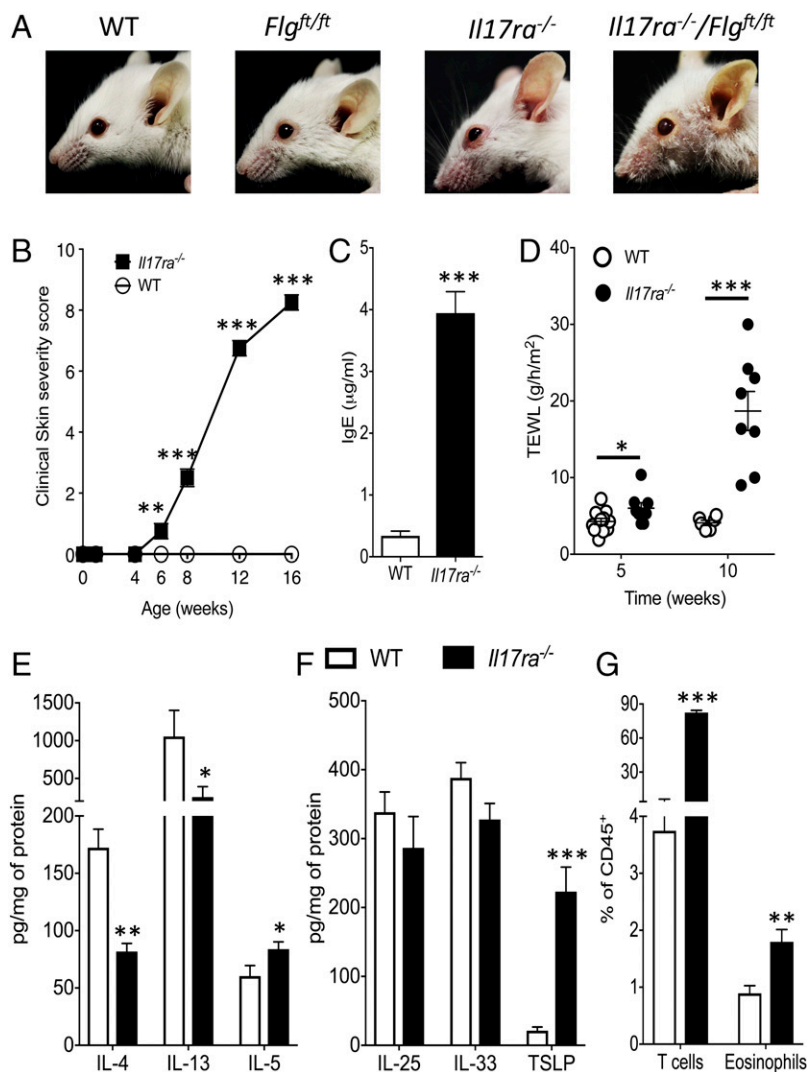


FIGURE 1. IL-17RA deficiency leads to spontaneous progressive allergic skin inflammation. **(A)** Representative images of 12-wk-old *Il17ra*^{-/-}/*Flg*^{fl/fl} and *Il17ra*^{-/-} mice compared with sex- and age-matched *Flg*^{fl/fl} and WT control mice. **(B)** Clinical skin severity score. **(C)** Total serum levels of IgE at 12 wk of age. **(D)** Measurement of TEWL of mice as indicated (*n* = 5–6 mice per group) at the specified time points. **(E)** Skin cytokine protein levels for IL-4, IL-13, and IL-5 prior to clinical skin inflammation, and **(F)** levels for TSLP, IL-33, and IL-25, determined by ELISA and expressed as picograms of cytokine per milligram of total skin protein. **(G)** Frequency of T cells and eosinophils in the skin expressed as a percentage of CD45⁺ cells. Data are presented as mean ± SEM, from four to nine mice per group, and statistical differences between groups were determined using a Student *t* test. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

chimeric mice. Irradiated WT mice that received bone marrow from *Il17ra*^{-/-} or WT donors did not develop skin inflammation, whereas *Il17ra*^{-/-} mice recipients of WT or *Il17ra*^{-/-}-derived bone marrow developed severe progressive skin inflammation (Fig. 2A, 2B). Furthermore, spontaneous serum IgE and increased Th2, Th17, and TCRγδ T cells in the skin-draining lymph nodes developed when IL-17RA-deficient mice were used as recipients irrespective of whether bone marrow cells were derived from WT or *Il17ra*^{-/-} donors (Fig. 2C–F). These data demonstrate that IL-17RA signaling on radioresistant skin-resident cells is required for the spontaneous development of allergic skin inflammation.

Skin inflammation in IL-17RA-deficient mice is independent of TCRγδ T cells and IL-22 but is dependent on CD4⁺ T cells and mediated by IL-5

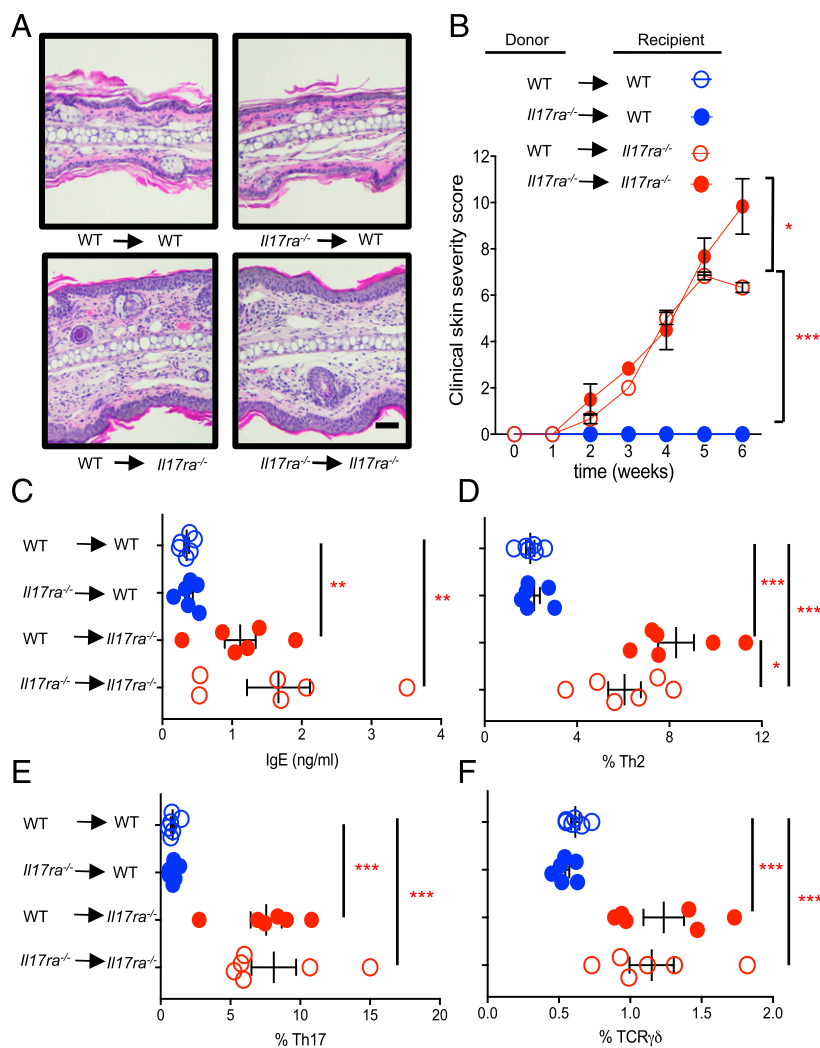
The increased numbers of TCRγδ T cells in *Il17ra*^{-/-} bone marrow chimeric mice (Fig. 2F) indicate a potential role for these cells in the spontaneous skin inflammation observed. Therefore, we further characterized TCRγδ T cells in *Il17ra*^{-/-} mice. TCRγδ T cells are significantly increased (*p* < 0.001) in *Il17ra*^{-/-} mice when compared with controls (Fig. 3A), an increase primarily due to infiltration of TCRγδ T cells with an activated tissue memory phenotype (data not shown). To comprehensively assess the role of TCRγδ T cells in the spontaneous skin inflammation developed by *Il17ra*^{-/-} mice, mice deficient in both TCRγδ T cells and IL-17RA (*Tcrδ*/*Il17ra*^{-/-}) were generated. TCRγδ T cell deficiency did not

prevent *Il17ra*^{-/-} mice from developing spontaneous skin inflammation (Fig. 3C).

Whereas IL-22 was not upregulated in the skin of *Il17ra*^{-/-} mice (Fig. 3B), it is widely implicated in mouse models of skin damage and inflammation (28), including skin inflammation downstream of IL-17RA signaling via Act1 (29). We therefore formally examined the contribution of IL-22 to the *Il17ra*^{-/-} skin phenotype by generating mice deficient in both IL-17RA and IL-22 (*Il17ra*/*Il22*^{-/-}). IL-22-deficient *Il17ra*^{-/-} mice developed spontaneous skin inflammation (Fig. 3C).

Although there is no role for TCRγδ T cells in the spontaneous skin phenotype (Fig. 3C), the marked T cell skin infiltration (Fig. 1G) suggests a role for adaptive immunity in the genesis of the skin inflammation. To formally address the contribution of adaptive immunity to the development of skin inflammation in the absence of IL-17RA signaling, we generated dual *Il17ra*^{-/-} and *Rag1*^{-/-} mice (*Il17ra*/*Rag1*^{-/-}). In the absence of T and B cells with ILCs present, *Il17ra*^{-/-} mice did not develop overt skin inflammation (Fig. 4A, 4B). However, reconstitution of *Il17ra*/*Rag1*^{-/-} mice with WT CD4⁺ T cells restored the development of spontaneous skin inflammation (Fig. 4C). As these data identify a central role for CD4⁺ cells in the development of type 2 skin inflammation due to IL-17RA deficiency, the frequency of IL-4-, IL-13-, and IL-5-expressing Th2 cells was examined in skin-draining lymph nodes from *Il17ra*^{-/-} mice and WT controls. The protein levels of IL-4

FIGURE 2. Absence of IL-17RA in the non-hematopoietic and not the hematopoietic compartment is required for spontaneous skin inflammation. **(A)** Representative images of H&E-stained ear tissue biopsies from sex- and age-matched bone marrow chimeric mice (bone marrow donor → recipient). Original magnification $\times 20$. Scale bar, 100 μm . **(B)** Clinical skin severity score (x -axis indicates time after bone marrow transfer). $n = 5$ mice per group. **(C)** Total serum IgE levels for the indicated bone marrow chimeric mice. **(D–F)** Flow cytometric analysis of Th2 (Gata3^{high}), Th17 (Roryt⁺), and TCR $\gamma\delta$ 17 (Roryt⁺) T cells for skin-draining lymph nodes of the indicated bone marrow chimeric mice. Each symbol represents individual mice ($n = 5$ mice per group). Data are presented as mean $\pm$ SEM, and statistical differences between groups were determined using a Student t test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



and IL-5 were significantly ($p < 0.05$ and $p < 0.01$, respectively) higher in the supernatants of in vitro-cultured lymph node cells derived from *Il17ra*^{-/-} mice compared with cells from WT mice (Fig. 4D). The frequency of IL-5-expressing CD4⁺ T cells was significantly ($p < 0.001$) increased in *Il17ra*^{-/-} skin when compared with controls, with no significant differences in the frequency of IL-4- or IL-13-expressing Th2 cells (Fig. 4E). We further characterized the IL-5-producing CD4⁺ T cells that were expanded in the skin of *Il17ra*^{-/-} mice. These skin-infiltrating IL-5⁺CD4⁺ T cells did not have increased IL-4 or IL-13 expression or CCR8 expression but had increased surface expression of T1/ST2 and CCR4 when compared with controls (Fig. 4F). The phenotype of these cells in IL-17RA-deficient mice is consistent with previously described IL-5⁺ peTh2 cells (30). Such peTh2 cell populations have been implicated in the pathogenesis of type 2 allergic diseases, including skin eosinophilia in patients with AD (31).

The increased levels of IL-5 in the skin of *Il17ra*^{-/-} mice and the infiltration of IL-5-expressing peTh2 cells in mice warranted further investigation of the role of CD4⁺ T cell-derived IL-5 in the development of spontaneous skin inflammation. CD4⁺ T cells from WT or IL-5-deficient (*Il5*^{cfp/cfp}) mice were transferred into *Il17ra*/Rag1^{-/-} mice, which do not develop skin inflammation (Fig. 4A, 4B), and the development of spontaneous skin inflammation in CD4⁺ T cell recipients was evaluated. Recipients of *Il5*^{cfp/cfp} CD4⁺ T cells had significantly ameliorated skin inflammation when compared with recipients of CD4⁺ T cells from WT

donors (Fig. 4G). Interestingly, recipients of CD4⁺ T cells from mice deficient in all four cardinal Th2 cytokines (IL-4, IL-5, IL-9, and IL-13) developed the same degree of protection as did recipients of IL-5-deficient CD4⁺ T cells, indicating a specific role for pathogenic IL-5-expressing CD4⁺ T cells in the spontaneous skin inflammation of the *Il17ra*^{-/-} mice (Fig. 4G). Furthermore, whereas transfer of WT CD4⁺ cells into *Il17ra*/Rag1^{-/-} mice induced a defective skin barrier, reflected in elevated TEWL, IL-5-deficient or IL-4/IL-5/IL-9/IL-13-deficient CD4⁺ T cells evoked significantly reduced skin barrier disruption (Fig. 4H). These studies identify a role for IL-5⁺ peTh2 cells in the generation of spontaneous skin inflammation in IL-17RA-deficient mice.

TSLP is required for skin inflammation in IL-17RA-deficient mice

Owing to the increased levels of TSLP in the skin of IL-17RA-deficient mice, we further examined the contribution of TSLP, as well as other skin alarmins, IL-25 and IL-33, in the development of spontaneous skin inflammation by generating mice deficient in both IL-17RA and TSLPR (*Il17ra*/Tslpr^{-/-}), or IL-25 receptor (*Il17ra*/Il17br^{-/-}) or IL-33 receptor (*Il17ra*/Il1rl1^{-/-}). TSLPR deficiency profoundly protected *Il17ra*^{-/-} mice from developing spontaneous skin inflammation (Fig. 5A, 5B). In contrast, *Il17ra*/Il17br^{-/-} and *Il17ra*/Il1rl1^{-/-} mice developed skin inflammation comparable to *Il17ra*^{-/-} mice (Fig. 5B), thus reinforcing the specific pathogenic role for TSLP in this model. In the absence of

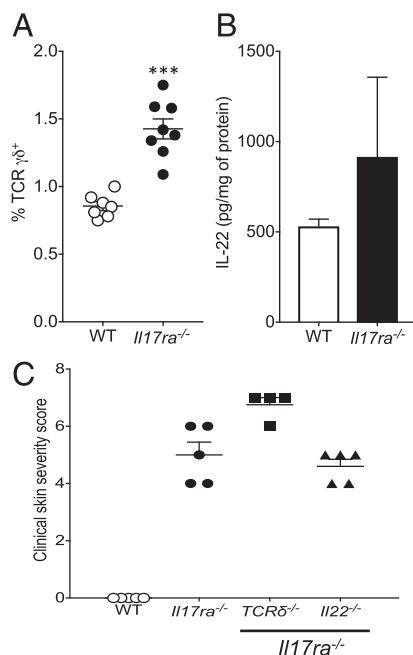


FIGURE 3. Skin inflammation in IL-17RA-deficient mice is independent of TCRγδ T cells and IL-22. **(A)** Flow cytometric analysis of TCRγδ⁺ T cells in WT and *Il17ra*^{-/-} mice (eight mice per group). **(B)** ELISA for the detection of IL-22 in the skin of WT and *Il17ra*^{-/-} mice (*n* = 3 mice per group). **(C)** Clinical skin severity score for the indicated sex- and age-matched mice (*n* = 3–5 mice per group). Data are presented as mean ± SEM, and statistical differences between groups were determined using a Student *t* test. ****p* < 0.001.

TSLP receptor signaling in IL-17RA-deficient mice, there were significantly reduced (*p* < 0.05) serum levels of IgE (Fig. 5C), ameliorated (*p* < 0.01) skin barrier disruption (Fig. 5D), and cell infiltration of the skin (Fig. 5E) was significantly reduced (*p* < 0.001). Additionally, the frequency of IL-5⁺ pTh2 cells in skin-draining lymph nodes was significantly (*p* < 0.005) reduced compared with *Il17ra*^{-/-} mice (Fig. 5F).

Skin microbiome dysbiosis in the absence of IL-17RA

Alterations in the skin microbiome are associated with AD and also a defective skin barrier (32, 33). 16S ribosomal sequencing analysis of skin from sex- and age-matched IL-17RA-deficient and WT mice revealed bacterial microbiome dysbiosis in the skin of *Il17ra*^{-/-} mice, with principal component analysis illustrating a dramatic segregation between the skin bacterial genera and also their relative abundances between the two groups (Fig. 6A). Bacterial taxonomic classification of the skin microbiome revealed an increase in bacteria of the Staphylococcaceae and Corynebacteriaceae families, which are associated with skin inflammation in AD in human and mouse models (33), in the skin of IL-17RA-deficient mice when compared with WT controls (Fig. 6B). Microbiome composition differences were also evident on the genus level with a significant (*p* < 0.05) increase in the abundance of the AD-associated *Corynebacterium*, *Staphylococcus*, and *Gemella* (Supplemental Fig. 4A). Further confirmation of microbiome dysbiosis in *Il17ra*^{-/-} mice was demonstrated by the significant amelioration (*p* < 0.0001) of skin inflammation in *Il17ra*^{-/-} mice following treatment of mice with a broad-spectrum antibiotic regimen (Fig. 6C). Consistent with pathogenic functions for IL-5 in the genesis of skin inflammation, antibiotic-treated *Il17ra*^{-/-} mice had significantly (*p* < 0.05) reduced circulating levels of IL-5 and, furthermore, also a significant reduction of pTh2 cells (*p* < 0.05; Figs. 6D, 7).

Owing to the microbiome dysbiosis and aberrant inflammation in the skin of *Il17ra*^{-/-} mice, the expression levels of a panel of antimicrobial peptides implicated in skin homeostasis was analyzed. The expression of S100 proteins (*S100a7*, *S100a8*, and *S100a9*), β-defensin 2 (*Defb2*) and cathelin-related antimicrobial peptide (*Cramp*) was assessed in the skin of *Il17ra*^{-/-} mice at 5 wk of age (no overt inflammation) and at 12 wk of age (inflamed skin) and compared with age- and sex-matched WT controls (Supplemental Fig. 4B). There were no differences between expression of selected antimicrobial peptide genes in the skin of 5-wk-old *Il17ra*^{-/-} mice and WT animals, whereas in the skin of 12-wk-old IL-17RA-deficient mice there was statistically reduced (*p* < 0.05) expression of *S100a8* and *S100a9* (Supplemental Fig. 4B). These data indicate changes in certain antimicrobial peptides in the skin of *Il17ra*^{-/-} mice only when the mice have developed skin inflammation. To address whether the dysbiotic microbiome of the IL-17RA-deficient mice is able to elicit spontaneous skin inflammation in WT mice, cohousing studies were undertaken. Antibiotic-treated WT mice that were cohoused with *Il17ra*^{-/-} mice with a dysbiotic microbiome did not develop spontaneous skin inflammation (Supplemental Fig. 4C). Furthermore, consistent with no skin inflammation, the levels of IL-5 and TSLP in the skin of WT antibiotic-treated mice cohoused with *Il17ra*^{-/-} or WT mice were comparable (data not shown). Collectively, these results show requirements for a dysbiotic microbiome, compromised skin barrier, and aberrant immunity for the development of spontaneous allergic-like skin inflammation due to IL-17RA deficiency.

Discussion

Dysregulation of the IL-17A/IL-17RA axis is implicated in inflammatory skin diseases, with Abs against IL-17A and IL-17RA showing significant efficacy for the treatment of psoriasis (3, 34). In AD patients, the increased expression of IL-17A in skin lesions raises the potential to target IL-17A or IL-17RA as a therapeutic strategy (4, 5). Additionally, absence of IL-17A has been shown to attenuate the development of skin inflammation in mouse models of AD (25). Although the functions of IL-17A signaling in skin inflammation are well investigated, a better understanding of the immunological function of IL-17RA in the skin is required. Our results highlight a previously unappreciated protective role for IL-17RA signaling in the maintenance of the integrity of the skin barrier and skin homeostasis. Interestingly, in the intestine IL-17RA signaling is implicated in the maintenance of the intestinal barrier via IL-17A-regulated expression of the tight junction protein occludin in the intestinal epithelial cells (35, 36). We now demonstrate that in mouse skin, keratinocyte IL-17RA signaling regulates the expression and processing of the skin barrier protein filaggrin, with absence of IL-17RA signaling creating an imbalance of monomeric filaggrin to profilaggrin composition of the skin leading to the subsequent skin barrier dysregulation. Therefore, in both the skin and gut IL-17RA signaling may be required for the preservation of barrier integrity and local immune homeostasis. However, although we report a role for IL-17RA signaling in maintenance of skin homeostasis, it remains to be determined in what degree IL-17A, IL-17F, IL-17A/F, or IL-17E contributes to the generation of skin inflammation. As Ab-based biologics against IL-17 family members are being developed, it will be interesting to determine whether alterations in barrier function, regulation of the microbiome or immunity in the skin, as addressed in this study, and also in the gut develop in patients with sustained blocking of IL-17RA or other IL-17 receptors and their ligands.

IL-17RA signaling may have regulatory roles in skin inflammation, as mice deficient in Act1, also on a BALB/c background as used in this study, which is downstream of IL-17RA and mediates

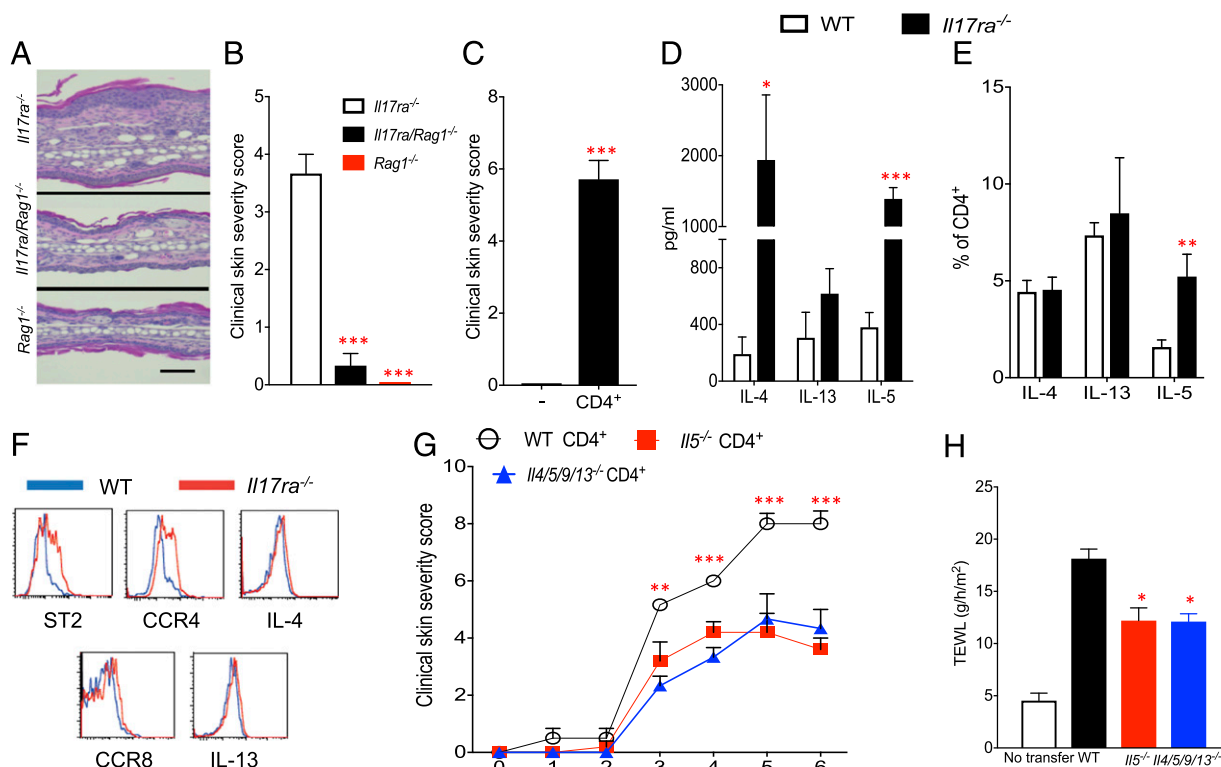


FIGURE 4. Skin inflammation in IL-17RA-deficient mice is CD4⁺ T cell-dependent and mediated by IL-5. (A) H&E-stained ear tissue biopsies from sex- and age-matched *Il17ra*^{-/-}, *Il17ra/Rag1*^{-/-}, and *Rag1*^{-/-} mice. Original magnification $\times 63$. Scale bar, 50 μ m. (B) Clinical skin severity score ($n = 4$ –5 mice per group). (C) Clinical skin severity score of *Il17ra/Rag1*^{-/-} mice following reconstitution with CD4⁺ T cells compared with nonreconstituted *Il17ra/Rag1*^{-/-} control mice. (D) ELISA for the detection of IL-4, IL-5, and IL-13 in the supernatant of in vitro-stimulated skin-draining lymph node cells of *Il17ra*^{-/-} and WT control mice (five mice per group). (E) Flow cytometric analysis for frequency of IL-4-, IL-13-, and IL-5-expressing CD4⁺ T cells in skin-draining lymph nodes (3–10 mice per group). (F) Flow cytometric analysis of IL-5-expressing CD4⁺ T cells from WT or *Il17ra*^{-/-} mice and expression of T1/ST2, CCR4, CCR8, IL-4, and IL-13. (G) Clinical skin severity score of *Il17ra/Rag1*^{-/-} mice reconstituted with CD4⁺ T cells from donor mice (three to six mice per group). (H) Measurement of TEWL of three to six mice per group. Data are presented as mean $\pm$ SEM and statistical differences between groups were determined using a Student *t* test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

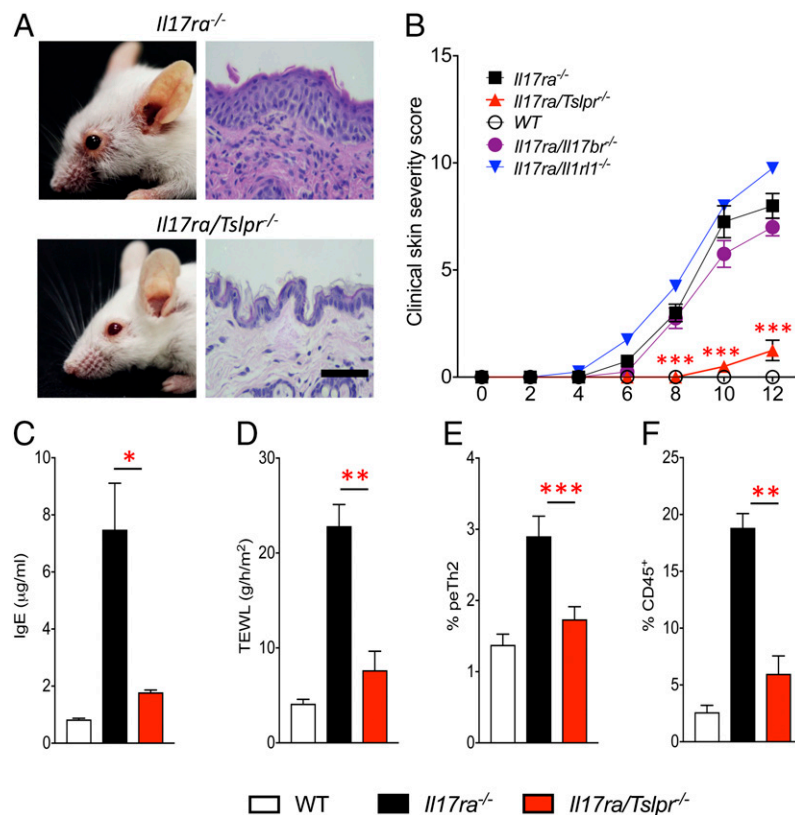
the subsequent activation of NF- κ B, develop spontaneous skin inflammation (29, 37). Hyperactive Th17 cells and primarily IL-22 were shown to be responsible for the spontaneous skin phenotype observed in these mice (29). In contrast to deficiency in Act1, in this study IL-17RA deficiency does not require IL-22 for the induction of spontaneous skin inflammation. This indicates that the genesis of skin inflammation due to Act1 deficiency and IL-17RA deficiency is mechanistically different. The use of bone marrow chimeras indicated that IL-17RA expression on skin radioresistant cells, such as epidermal Langerhans cells and keratinocytes, was responsible for the development of the AD-like skin inflammation. In a mouse model of IL-17A and IL-17C psoriatic skin inflammation it was also recently reported that disease was mediated by nonhematopoietic cells (27), with the same IL-17A-expressing cellular compartment responsible for synovitis in a model of joint inflammation (38). These studies highlight a specific role for IL-17RA expression on nonhematopoietic cells in the pathogenesis of inflammation in different tissues.

In this study, IL-17RA-deficient mice on the BALB/c mouse strain, commonly used for the study of allergic diseases (39), developed marked spontaneous skin inflammation, that is, without the need for skin insult and barrier disruption. Most available studies in the public domain using IL-17RA signaling-deficient mice involve the C57BL/6 background with no reports of a spontaneous skin inflammatory phenotype. However, IL-17RA-deficient mice on a C57BL/6 background also have a dramatically

reduced threshold for the development of skin inflammation following insult with the chemical agent MC903, and they develop severely accelerated skin inflammation. It is relevant that in mice with a defective skin barrier due to a filaggrin mutation, there is also more exacerbated disease when on a BALB/c background but not on a C57BL/6 background (10). Similar to human AD, the development of skin inflammation due to IL-17RA deficiency in mice may also be a polygenic condition, with mouse strain genetic differences, as well as impact of animal breeding environment, influencing the magnitude of skin dysregulation and resulting inflammation.

In IL-17RA-deficient mice skin inflammation was characterized by elevated IL-5 in this skin, with CD4⁺ T cell transfer showing that inflammation was IL-5-dependent. In patients with AD, polymorphisms in the IL-5 encoding gene have previously been associated with disease (40), whereas changes in IL-5 serum levels have been shown to reflect clinical improvement of AD in patients (41). Recently emerging evidence implicates IL-5⁺-producing pTh2 cells in the initiation of chronic inflammatory diseases, including AD (42). IL-5-producing pTh2 cells drive eosinophilic airway inflammation and skin eosinophilia, leading to tissue damage and chronicity of the immune response, and they have been shown in both human and mouse models (30, 31). Little is known, however, regarding the regulation of these cells and the mechanisms that influence their expansion. In this study, we reported that IL-17RA deficiency induced an increase in pTh2 T cells that was associated with spontaneous skin inflammation

FIGURE 5. TSLP is required for skin inflammation in IL-17RA-deficient mice. **(A)** H&E-stained skin tissue biopsies from sex- and age-matched *Il17ra*^{-/-} and *Il17ra/Tslpr*^{-/-} mice. Original magnification $\times 63$. Scale bar, 50 μ m. **(B)** Clinical skin severity score of *Il17ra*^{-/-}, *Il17ra/Tslpr*^{-/-}, *Il17ra/Il17br*^{-/-}, *Il17ra/Il1rl1*^{-/-}, and WT control mice ($n = 4$ –6 mice per group). **(C)** Total serum levels of IgE. **(D)** Measurement of TEWL of mice as indicated, compared with sex- and age-matched controls ($n = 3$ –5 mice per group). **(E)** Frequency of infiltrating cells to the skin ($n = 7$ –9 mice per group). **(F)** Flow cytometry analysis of IL-5-expressing CD4⁺ pTh2 in skin-draining lymph nodes ($n = 7$ –9 mice per group). Data are presented as mean $\pm$ SEM, and statistical differences between groups were determined using a Student *t* test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



and skin eosinophilia, and importantly we demonstrated a specific role for IL-5. Although AD patients treated with a humanized IL-5-neutralizing Ab showed only modest improvement, further study into the contribution of IL-5 in AD is warranted (43). Although our data show a role for IL-5 in the development of skin inflammation, it is clear that other IL-5-independent mechanisms may also be involved. However, whereas IL-5 deficiency reduced the severity of skin inflammation, TSLP was mandatory for the development of AD-like inflammation in IL-17RA-deficient mice. These data raise the potential of exploring combined biologic

targeting of the alarmin initiator and cytokine effector, in this case TSLP and IL-5 for AD, respectively, as a novel bispecific therapeutic strategy for inflammatory diseases.

Several studies have implicated TSLP in atopic diseases, including AD (44). TSLP is primarily expressed by epithelial cells at barrier surfaces, including skin keratinocytes (45). Keratinocyte-specific overexpression of TSLP in mice has previously been shown to induce spontaneous skin inflammation with several characteristics of AD (46). Interestingly IL-17A inhibits secretion of TSLP by human and mouse keratinocytes (47) (data not shown), and

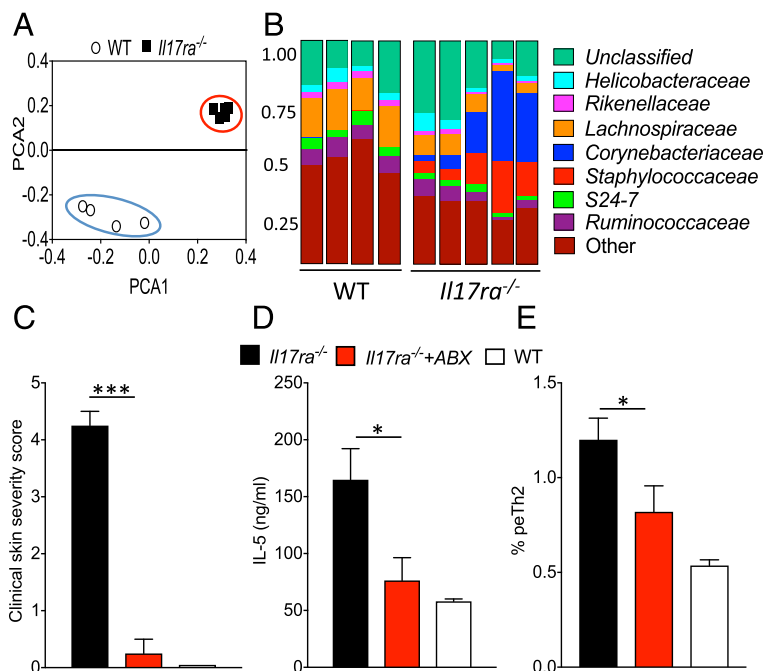
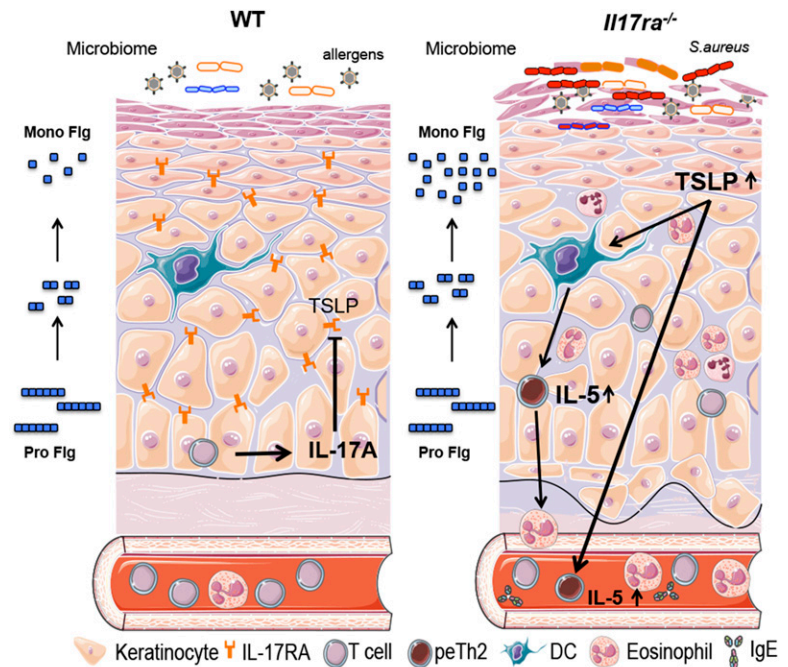


FIGURE 6. Skin microbiome dysbiosis in the absence of IL-17RA and antibiotic treatment attenuated skin inflammation. **(A)** Principal component analysis of skin microbiome samples isolated from *Il17ra*^{-/-} and sex- and age-matched WT control mice. **(B)** Bacterial families identified on the skin of *Il17ra*^{-/-} and WT mice; mean relative abundance is shown. **(C)** Clinical skin severity score of indicated groups of mice following treatment with an antibiotic (ABX) regimen or treatment with autoclaved water ($n = 5$ mice per group). Serum levels of IL-5 **(D)** and frequency of IL-5-expressing CD4⁺ pTh2 **(E)** cells in skin-draining lymph nodes ($n = 5$ mice per group). Data are presented as mean $\pm$ SEM, and statistical differences between groups were determined using a Student *t* test. * $p < 0.05$, *** $p < 0.001$.

FIGURE 7. Schematic of the aberrant skin barrier and development of allergic cutaneous inflammation in the absence of IL-17RA signaling.



therefore absence of IL-17A-mediated inhibitory signals due to an IL-17RA deficiency could be a major confounding factor for the high levels of TSLP expression observed in IL-17RA-deficient mice. Although TSLP can induce type 2 responses (48) and can elicit allergic skin inflammation via ILC2 and/or basophils (49, 50), both ILC2 and basophils were not altered in the skin of IL-17RA-deficient mice (data not shown). Another mechanism for TSLP-initiated skin inflammation involves TSLP-activating dendritic cells to drive naive T cell polarization to IL-4-expressing Th2 cells (48). Indeed, keratinocyte-derived TSLP has been shown to act on skin Langerhans cells, which respond to TSLP to elicit strong Th2 responses in a model of allergic skin dermatitis (51). It remains to be elucidated how TSLP is contributing to the spontaneous skin inflammation observed in IL-17RA-deficient mice.

An important role of IL-17RA in skin microbiome maintenance was shown by mice deficient in IL-17RA signaling developing microbiome dysbiosis. Furthermore, the skin inflammation in IL-17RA-deficient mice was ameliorated by antibiotic intervention, supporting the premise that the aberrant microbiome directly contributes to the skin inflammation. However, microbiome transfer cohousing studies indicated that the dysbiotic microbiome of IL-17RA-deficient mice is, in isolation, not adequate to lead to spontaneous skin inflammation when transferred to WT mice that have an intact skin barrier. In various inflammatory skin diseases, including AD, there are alterations in the expression of antimicrobial peptides in the skin that may function in cutaneous defense as well as contribute to pathogenesis (52, 53). In 5-wk-old IL-17RA-deficient animals, when the skin has no overt inflammation, the expression of *S100a7*, *S100a8*, *S100a9*, *Defb2*, and *Cramp* was comparable to that of WT mice. In older *Il17ra*^{-/-} mice with inflamed skin, although there was no change in expression of *S100a7*, *Defb2*, and *Cramp*, there was reduced expression of *S100a8* and *S100a9*, two antimicrobial peptides implicated in AD and activated by IL-17A (54). Although it remains to be determined what the initiator of the spontaneous skin inflammation in the absence of IL-17RA signaling is, this study highlights the intricate interplay of the triad of a defective skin barrier, aberrant inflammation, and microbial dysbiosis in skin function in health and disease.

Skin microbiome dysbiosis has previously been associated with AD in humans and mice (32, 33). Such is the contribution of the

skin microbiome in AD that age-related differences in disease prevalence could be a result of composition differences of the microbiome in pediatric and adult AD (55). Disease severity and disease flares show strong association with changes in microbiome diversity in patients with AD, with particularly strong correlation between severity and bacteria of the *Staphylococcus*, *Corynebacterium*, and *Gemella* genera (33, 56). Interestingly, the genera *Staphylococcus* and *Corynebacterium* were both increased in the skin of IL-17RA-deficient mice in this study. Although this is a mouse study, intriguingly, humans carrying mutations leading to IL-17RA signaling deficiency develop chronic mucocutaneous candidiasis and, as seen in the present study, also may develop additional staphylococcal infections of the skin as well as eczema (57).

Collectively, these results reveal that dysregulation of the skin in *Il17ra*^{-/-} mice involves skin microbiome dysbiosis, a defective skin barrier with alteration in filaggrin expression, and aberrant skin inflammation mediated by IL-5 and peTh2 cells and dependent on TSLP (Fig. 7). This study advances our current understanding of the regulatory role of IL-17RA in maintaining the skin barrier and immune system homeostasis to prevent the spontaneous development of allergic dermatitis.

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Disclosures

The authors have no financial conflicts of interest.

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Fig. S1

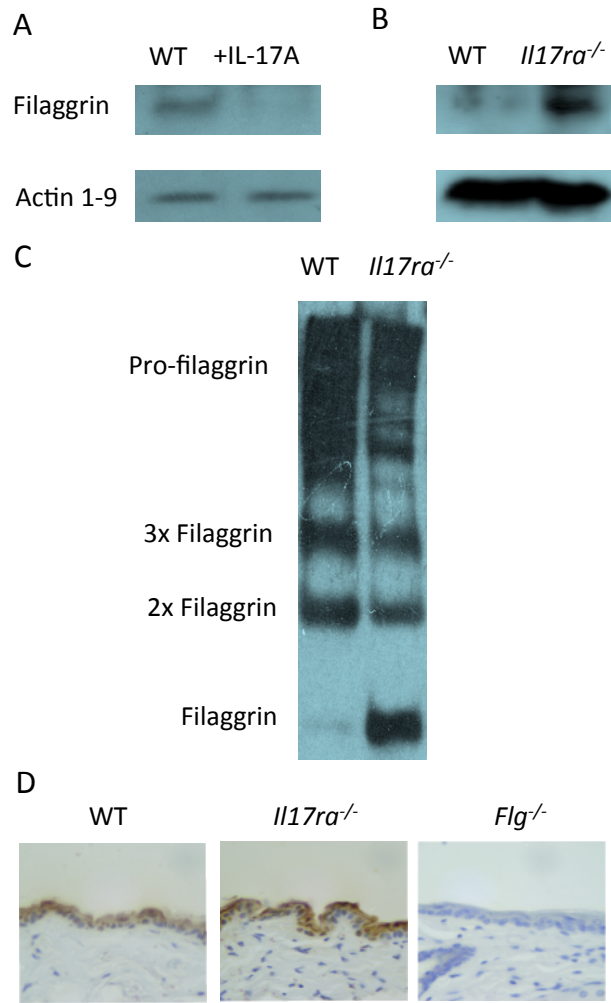


Fig. S1. Altered filaggrin expression in the skin of *Il17ra*^{-/-} mice

A. Western blot analysis of keratinocytes isolated from WT mice, following *ex vivo* differentiation and treatment with IL-17A, for expression of filaggrin monomer (data shown representative of 2 independent experiments). **B.** Western blot analysis of keratinocytes isolated from WT and *Il17ra*^{-/-} mice following *ex vivo* differentiation for expression of filaggrin monomer (data shown representative of 2 independent experiments). **C.** Western blot analysis for filaggrin expression in epidermal skin of non-inflamed 5-week old *Il17ra*^{-/-} mice and sex- and age-matched WT control mice, a total of 5 mice per group were analysed with similar results. **D.** Representative immunohistochemistry images for filaggrin staining of skin biopsies from non-inflamed 5-week old *Il17ra*^{-/-} compared to sex- and age-matched WT control mice. *Flg*^{-/-} mouse skin biopsies were used as negative controls. Images are representative of at least 3 mice per group (magnification x63).

Fig. S2

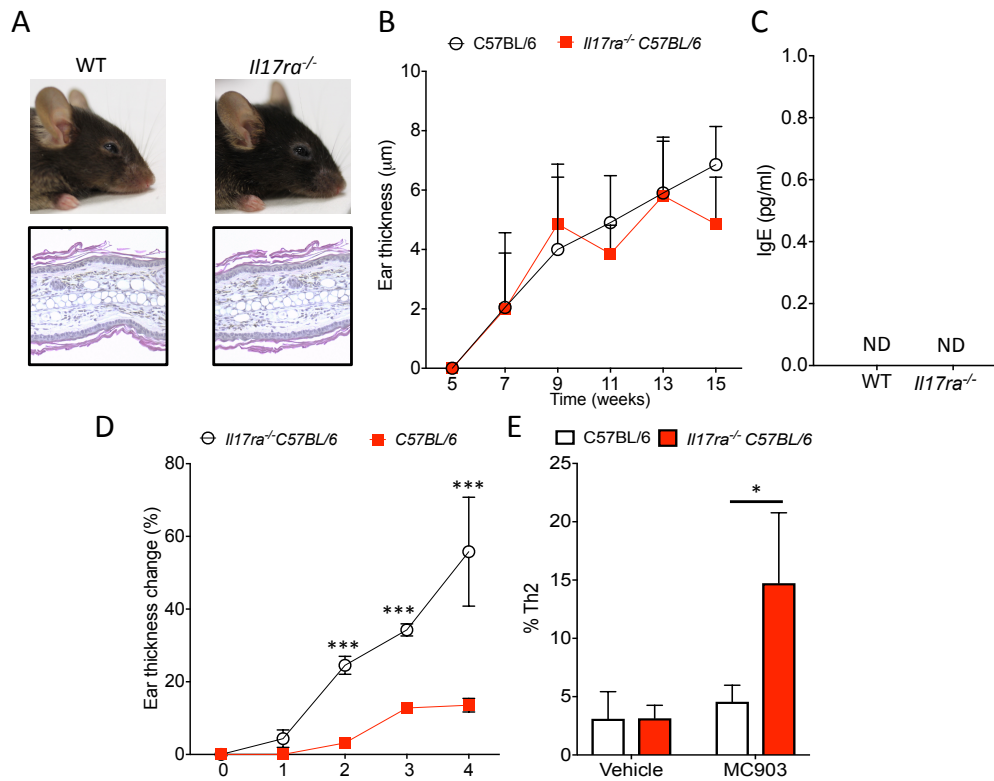


Fig. S2. No spontaneous skin phenotype but increased inducible inflammation in *Il17ra*^{-/-} mice on a C57BL/6 background.

A. Representative images of IL-17RA deficient C57BL/6 mouse with no skin inflammation and WT mouse. H&E-stained section of ear from sex- and age-matched *Il17ra*^{-/-} mice on C57BL/6 background and control C57BL/6 mice (original magnification x63). **B.** Ear thickness of indicated mice over time (3-5 mice per group). **C.** Serum IgE levels for the indicated mice (3-5 mice per group, 15-weeks of age) **D.** Ear thickness changes over time following topical treatment with MC903 to *Il17ra*^{-/-} mice on C57BL/6 background and WT controls (3-5 mice per group). **E.** Frequency of Th2 cells (CD4⁺Gata3^{high}) in skin draining lymph nodes in mice with or without treatment with MC903 (3-5 mice per group). Data are presented as mean and SEM and statistical differences between groups were determined using Student's *t* test.

Fig. S3

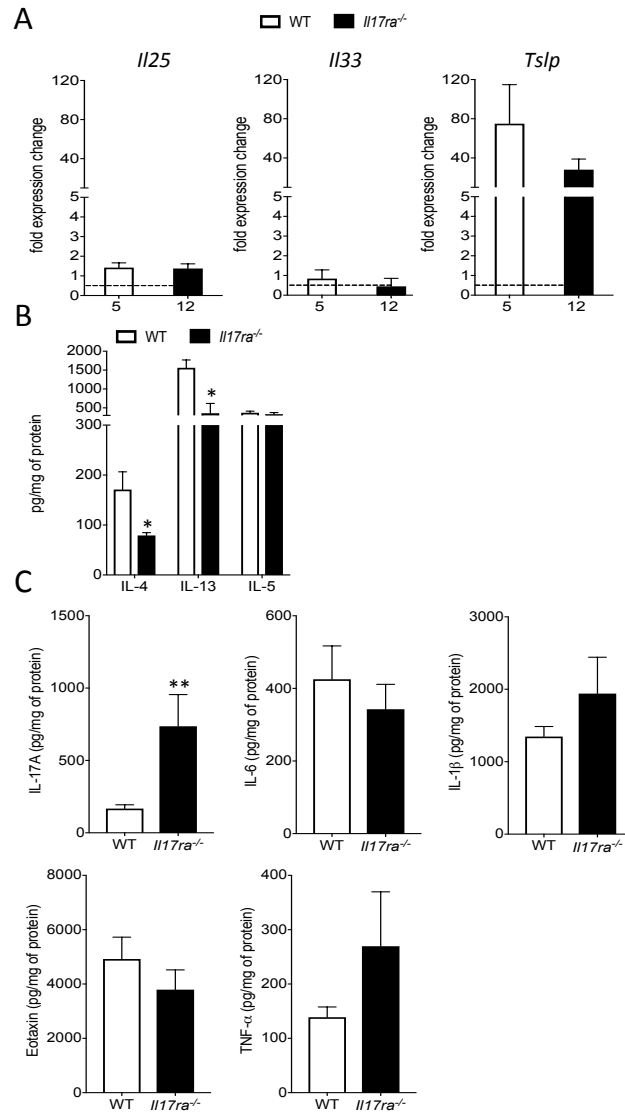


Fig. S3. Cytokine expression in skin of *Il17ra*^{-/-} mice and control animals.

A. qPCR for the expression of the alarmins TSLP, IL-25 and IL-33 in the skin of *Il17ra*^{-/-} mice before (5 weeks of age) and after (12 weeks of age) the development of spontaneous skin inflammation, data presented as fold expression change of *Il17ra*^{-/-} mice over age- and sex-matched WT controls (3-5 mice per group). **B, C.** Protein levels for the indicated cytokines in skin of *Il17ra*^{-/-} mice and sex- and age-matched WT controls (12 week-old, 3-5 mice/group). Data are presented as mean and SEM and statistical differences between groups were determined using Student's *t* test.

Fig. S4

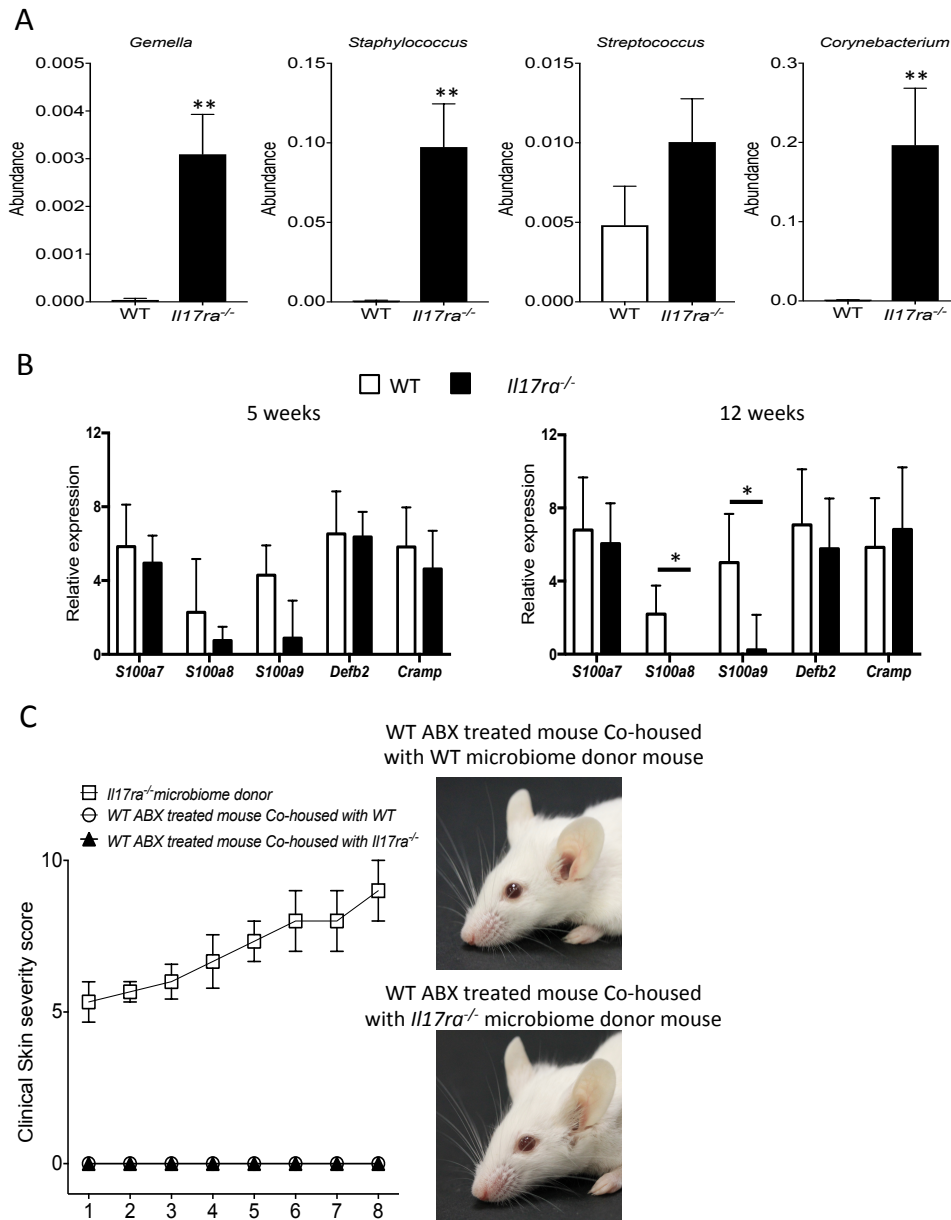


Fig. S4. Microbiome dysbiosis, skin AMP expression and co-housing studies.

A. Abundance of bacterial genera identified on the skin of *Il17ra*^{-/-} and WT mice (4-5 mice/group). **B.** qPCR for the relative expression of antimicrobial peptide genes in the skin of *Il17ra*^{-/-} mice before (5-weeks of age) and after (12-weeks of age) the development of spontaneous skin inflammation and expression in skin of age- and sex-matched wild type (WT) mice (3-4 mice per group). **C.** Clinical skin severity score and representative images of antibiotic (ABX)-treated WT mice following co-housing with the indicated microbiome donor mice for 8 weeks. Data are presented as mean and SEM and statistical differences between groups was determined using Student's *t* test.