Neutralization of IL-17C Reduces Skin Inflammation in Mouse Models of Psoriasis and Atopic Dermatitis

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IL-17C is a functionally distinct member of the IL-17 family that was believed to play a role in the pathogenesis of psoriasis. Here we confirmed that IL-17C is involved in psoriasis and explored potential roles for IL-17C in atopic dermatitis (AD). An anti-IL-17C antibody, MOR106, was generated that potently and selectively binds to human and mouse IL-17C, thereby inhibiting the binding of IL-17C to its IL-17RE receptor. The antibody inhibited cutaneous inflammation in an IL-23–induced psoriatic-like skin inflammation model. In lesional skin of patients with AD, IL-17C expression levels were increased and localized to keratinocytes and infiltrating immune cells. To determine the contribution of IL-17C to AD pathogenesis, MOR106 was tested in two distinct in vivo models. In the calcipotriol-induced AD model, ear skin inflammation, TSLP, and IL-33 protein production in ears was suppressed by MOR106. Consistently, in the flaky tail strain mouse model, spontaneous development of AD-like skin inflammation was reduced by MOR106. Moreover, serum IgE levels, number of mast cells in skin and T helper type 2-related cytokines IL-4 and CCL17 in serum were all reduced. Overall, our results indicate that IL-17C is a central mediator of skin inflammation beyond psoriasis and is relevant in particular in AD.


INTRODUCTION

IL-17C was originally discovered by Li et al. (Li et al., 2000) and represents one of the six members of the IL-17 cytokine family, which consists of IL-17A through IL-17F. IL-17C shows low sequence identity (23%) with its best characterized family member, IL-17A. The IL-17C receptor is a heterodimer composed of the IL-17RA and IL-17RE subunits. The IL-17RE subunit confers binding specificity for IL-17C (Chang et al., 2011). Intracellular signaling of the IL-17RA/RE complex activates the NF-kB and mitogen-activated kinase pathways (Chang et al., 2011; Song et al., 2011). In contrast to IL-17A, which is produced by T helper type (Th) 17 cells and innate immune cells such as γδ T and invariant natural killer T cells (Cua and Tato, 2010), the main source for IL-17C appears to be epithelial cells (Ramirez-Carrozzi et al., 2011; Song et al., 2011).

IL-17C expression is induced in cultured epithelial cells by proinflammatory cytokines (TNF-α and IL-1β) or bacterial stimuli, via TLR2/5 (Johansen et al., 2011; Ramirez-Carrozzi et al., 2011). Expression of the IL-17RE/IL-17RA complex has been mainly shown on epithelial cells, including keratinocytes and colon epithelial cells. The receptor expression on epithelial cells would allow for an autocrine stimulation loop inducing expression of other proinflammatory mediators and antimicrobial peptides in epidermal keratinocytes (Ramirez-Carrozzi et al., 2011). In addition, the ability to synergize with other cytokines (e.g., TNF-α) represents another way for IL-17C to amplify inflammatory responses (Johnston et al., 2013).

IL-17C has been suggested to play a role in various inflammatory pathologies, such as psoriasis and lung inflammation, and in the experimental autoimmune encephalomyelitis model of multiple sclerosis (Chang et al., 2011; Johnston et al., 2013; Pleifert et al., 2013; Ramirez-Carrozzi et al., 2011). In particular in psoriasis, evidence has accumulated implicating IL-17C as the proinflammatory cytokine driving disease activity. Increased IL-17C expression was shown at the RNA level in lesional psoriatic skin compared with nonlesional skin (Johansen et al., 2009). Consistently, IL-17C protein levels have been measured to be approximately 3 to 4 times greater in lesional versus nonlesional psoriatic skin compared with nonlesional skin (Johansen et al., 2009; Johnston et al., 2013). In biopsy samples from psoriasis patients, IL-17C localized to keratinocytes and was found to some extent in endothelial cells and infiltrating immune cells (Johnston et al., 2011).
et al., 2013). In animal models, intradermal injections of IL-17C led to epidermal thickening (Ramirez-Carrozzi et al., 2011). Similarly, transgenic keratin 5–IL-17C mice overexpressing IL-17C in keratinocytes developed skin inflammation and spontaneous psoriatic-like lesions (Johnston et al., 2013). Consistent with a role for IL-17C in the generation of psoriasis, the development of psoriasis-like skin inflammation by imiquimod was significantly reduced in IL-17C− or IL-17RE−knockout mice (Ramirez-Carrozzi et al., 2011).

In psoriasis, inflammatory myeloid dendritic cells are thought to produce IL-23 to subsequently induce the formation and activation of Th17 and Th22 cells, which on their part secrete IL-17A, IFN-γ, TNF-α, and IL-22. These cytokines activate dermal keratinocytes, amplifying the psoriatic inflammation (Lowes et al., 2014). In contrast to the Th17-driven disease psoriasis, atopoid dermatitis (AD) is characterized by epidermal barrier damage, which may be caused either by an underlying genetic background or by external triggers, as well as by immune alterations characterized by type 2 immunity. More recently, there is growing recognition that AD is not only associated with Th2 immunity but that Th17 and Th22 inflammatory responses are also implicated (Noda et al., 2015; Weidinger and Novak, 2016).

Several animal models reproducing certain aspects of AD have been described (Jin et al., 2009). In the model introduced by Li et al. (2006, 2009), topical administration of a vitamin D3 analog, MC903 (calcipotriol), induces AD-like skin lesions characterized by red and scaly skin and accompanied by epidermal hyperplasia and dermal infiltration of various cell types, as well as an increase of Th2 cytokines in the skin and elevated serum IgE level. An alternative model of AD is the flaky tail mutant mouse (Matt+matflgth), which exhibits a defective skin barrier due to mutations in the Matt and filagrin genes, spontaneously developing atopy and progressive overt dermatitis (Fallon et al., 2009; Sasaki et al., 2013; Saunders et al., 2013).

We hypothesized that IL-17C, as a local amplifier of ongoing skin inflammation, can drive disease activity independently of the underlying type of adaptive immunity and is expected to play a role in other skin inflammatory diseases beyond psoriasis. Here we confirmed the role of IL-17C in psoriasis by testing an IL-17C–neutralizing antibody in the IL-23–induced psoriasis-like skin model and further evaluated the role in the development of AD by testing the antibody in two distinct in vivo models.

**RESULTS**

**IL-17C expression is increased in the skin of AD patients**

Several lines of evidence point toward a role for IL-17C in driving disease activity in psoriasis. We hypothesized that IL-17C might play a role in other inflammatory skin diseases, such as AD. We first investigated the expression of IL-17C in human AD skin samples and in psoriatic skin. Compared with noninvolved autologous skin from the same patient, IL-17C mRNA was up-regulated in AD lesions to similar levels to those found in psoriatic skin (Figure 1a). Furthermore, immunohistochemistry analysis showed that IL-17C expression in the lesional skin of psoriatic and AD patients, compared with the skin of healthy subjects, was increased in the keratinocytes and was also detected in infiltrating immune cells in the dermis (Figure 1b).

**MOR106 antibody potently binds IL-17C and blocks IL-17C activity in vitro**

Targeting the IL-17C/IL-17RE pathway with a specific antagonist has not been reported yet. A neutralizing IL-17C antibody was generated to investigate the role of IL-17C in relevant animal models. The MOR106 antibody was identified from the MorphoSys Ylanthia library (Tiller et al., 2013) after solution pannings with mouse and human IL-17C were applied, followed by in vitro maturation using diversified complementarity-determining region (ie, the antibody part that dictates binding to the antigen) modules to optimize binding properties. MOR106 is a human IgG1 that binds both human and mouse IL-17C. No binding was observed to other human and mouse IL-17 family members (Figure 2a). MOR106 potently blocked the interaction of human and mouse IL-17C with its high affinity receptor IL-17RE (Figure 2b). MOR106 reduced NF-κB reporter activation stimulated by human and mouse IL-17C in mouse fibroblasts transfected with the mouse IL-17RE subunit (Figure 2c). Blocking of functional activity was also shown on primary keratinocytes that endogenously express the IL-17RA/RE receptor complex. Stimulation with IL-17C and TNF-α enhanced the expression of DEFB4A in human keratinocytes and of CSF3 in mouse keratinocytes. MOR106 effectively reduced DEFB4A (human) or CSF3 (mouse) gene expression, thus confirming functional neutralization of IL-17C–mediated responses in both human and mouse keratinocytes (Figure 2d).

**Neutralization of IL-17C attenuates development of psoriasis-like skin inflammation in the IL-23 injection model**

Data obtained with IL-17C−knockout and transgenic mice have shown the pathogenic role of this cytokine in animal models of psoriasis. To validate the role of IL-17C in a Th17-driven disease and the functionality of our antibody in vivo, we evaluated MOR106 in the IL-23 intradermal injection model of psoriatic skin inflammation. In this model, the expression of IL-17C is increased in inflamed skin (see Supplementary Figure S1 online), and treatment of mice with MOR106 reduced the development of IL-23–induced skin inflammation, as evidenced by significantly lowered ear swelling and acanthosis (Figure 3a and b). Furthermore, gene expression of IL-23–induced cytokines in the skin, including IL-17A, IL-22, IL-1β, S100A9/8, and LCN2, were reduced by anti-IL-17C mAb treatment (Figure 3c). Overall, these data show that MOR106 neutralizes IL-17C in vivo to suppress the development of experimental psoriasis and that this antibody can be used in other animal models of inflammatory diseases to further explore the role of IL-17C.

**IL-17C contributes to the development of AD-like skin lesion in MC903 model**

Topical application of the low-calcemic vitamin D3 analog MC903 is described to induce changes in mouse skin morphology and inflammation resembling immune perturbations observed in acute lesions of patients with AD. IL-17C protein was significantly up-regulated in skin of MC903-treated mice, as measured by immunohistochemistry.
increased skin IL-17C expression plays a significant role in barrier function (Figure 4 f). In conclusion, the data show that functions—for example, Th1-, Th2-, and Th17-related were observed across gene sets related to different biological real-time PCR (qPCR) (see Supplementary Figure S3 online), effects, some of which were further confirmed by quantitative multiple AD-related genes by MOR106 treatment. Clear effect observed in MC903-treated skin was reverted for transcriptomic analysis. The disease-related gene expression AD-like inflammation model, we performed a microarray the inflammatory pathways affected by IL-17C in the MC903 (Li et al., 2017 ). Analysis of protein expression levels is known to depend on TSLP (Li et al., 2009 ) and IL-33 a cytokine that controls migration of Th2 lymphocytes, and treatment of mice led to increases in plasma levels of CCL17, both the epidermal and dermal layers (Figure 4 c). MC903 higher significantly prevented the increase in thickness of by histomorphometry. MOR106 at doses of 10 mg/kg or MOR106, epidermal and dermal thicknesses were evaluated (Figure 4 b). To characterize the reduction in ear thickness by MOR106, edematous and dermal thicknesses were evaluated by histomorphometry. MOR106 at doses of 10 mg/kg or significantly prevented the increase in thickness of both the epidermal and dermal layers (Figure 4c). MC903 treatment of mice led to increases in plasma levels of CCL17, a cytokine that controls migration of Th2 lymphocytes, and CCL17 was reduced upon treatment with MOR106 increased IL-17C levels in lesional skin (see Supplementary (Figure 5 c) in animals treated with 30 mg/kg of 17C antibody in the flaky tail mutant mouse strain of spontaneous AD-like disease. At the start of treatment, the flaky tail mice already showed signs of spontaneous eczematous-like dermatitis, reflected in a mild clinical score. This established skin inflammation further progressed during the 6-week observation period to overt dermatitis in isotype-treated mice (Figure 5 a) with increased IL-17C levels in lesional skin (see Supplementary Figure S4 online). MOR106 reduced the progression of the skin severity score with a trend for 3 mg/kg and a significant suppression of skin inflammation, comparable to the effect of dexamethasone, observed at 30 mg/kg (Figure 5 a). Observation of the dorsal, ventral, and neck regions clearly showed the improvement caused by anti-IL17C treatment, with animals having less hair loss on the shoulder region and less skin erythema and excoriation under the neck (see Supplementary Figure S5 online). An amelioration of inflammation with the MOR106 antibody was also observed at the level of blepharitis assessed at the end of the treatment (see Supplementary Figure S6 online). Consistent with the reduced skin inflammation in anti-IL-17C–treated mice, histomorphometric analysis of lesional skin showed a small but significant reduction of acanthosis upon treatment with MOR106 at the dose of 30 mg/kg (Figure 5 b). NO differences were detected in any of the treatment groups concerning number of eosinophils or lymphocytes (data not shown). In contrast, a small but significant effect was observed on the number of mast cells in the skin of MOR106-treated mice, which was similar to levels in dexamethasone-treated animals (Figure 5 b). Flaky tail mice have an elevation in serum IgE as the spontaneous development of skin inflammation progresses, and these levels were significantly reduced (Figure 5c) in animals treated with 30 mg/kg MOR106. In addition, serum Th17-, Th2-, and Th1-related cytokines/chemokines in flaky tail mice were measured by
ELISA and compared with those of wild-type mice. Serum cytokine levels were generally low; however, IL-4, IL-17A, and CCL17 were significantly up-regulated, whereas IFN-γ was not significantly modulated (Figure 5d), confirming the reported Th2/Th17-dominant cytokine profile in this model. MOR106 treatment significantly reduced the levels of IL-4 and CCL17, similar to dexamethasone, but did not significantly affect serum IL-17A levels, suggesting that IL-17C mainly affects the Th2 axis in this model (Figure 5d). Collectively, these data show that targeting IL-17C with MOR106 significantly attenuated the development of established AD-like inflammation in the flaky tail model of spontaneous dermatitis.

**DISCUSSION**

Based on the described role of IL-17C in psoriasis, we postulated that IL-17C, as a local amplifier of skin inflammation, could also play a role in other skin inflammatory diseases. In this study, we examined the role of IL-17C in AD.

We observed IL-17C expression in keratinocytes and infiltrating immune cells in AD and psoriatic skin, which is consistent with earlier observations reported for psoriatic skin (Johnston et al., 2013). Furthermore, increased production of IL-17C protein in AD patients compared with healthy subjects has been observed in a phase 1 trial of MOR106 (NCT02739009) (unpublished data). Until now, the biological function of IL-17C in vivo was studied in knockout animals carrying a genetic deletion of the gene or overexpressing IL-17C (Johnston et al., 2013; Ramirez-Carrozzi et al., 2011; Song et al., 2011). We used a potent IL-17C-neutralizing antibody to study the role of this cytokine in the context of cutaneous inflammation in vivo. Three animal models of skin inflammation were investigated, each reflecting different aspects of immunological perturbation. The hyperplasia induced in the IL-23 dermal injection model is described to be primarily driven by Th17 via production of IL-17A and IL-22 as downstream effector cytokines (Rizzo et al., 2011). Conversely, in the MC903 model, application of calcipotriol triggers production of keratinocyte TSLP and IL-33 which induces the barrier impairment essential for generating a subsequent Th2 allergic immune response (Leiva-Castillo et al., 2013; Li et al., 2006; Li et al., 2017). Finally, rather than addressing inducible skin inflammation, the dysregulated skin barrier function in the flaky tail model facilitates allergenic sensitization, leading to the spontaneous eczematous inflammation characterized by a generalized inflammatory response in lesional skin evidenced by increased type 2 and type 17 cytokines (Fallon et al., 2009; Saunders et al., 2013). The IL-17C-neutralizing antibody MOR106 showed amelioration of disease-associated parameters in all three models of skin inflammation, implying that IL-17C is a key driver of skin inflammation.

IL-17C appears to differ from classical T-cell cytokines, because it is believed to be produced predominantly by epithelial cells, such as skin keratinocytes. In addition, the IL-17RA/RE receptor, also expressed on keratinocytes, can trigger an autocrine loop of stimulation (Ramirez-Carrozzi et al., 2011). We propose that in the skin, IL-17C acts as an
activating stimulus on keratinocytes, which is supported by the finding of reduced TSLP, IL-33, and/or CCL17 levels in both the MC903 and the flaky tail models. The particular autocrine action of IL-17C at the level of keratinocytes provides an explanation of why an anti-IL-17C antibody can have anti-inflammatory properties in both Th17- and Th2-driven inflammatory skin inflammation. In line with this hypothesis, transcriptomic analyses showed that IL-17C blockade counteracts MC903-induced modulation of Th1-, Th2-, and Th17-related genes, indicating a central role for IL-17C in T-cell regulation upon MC903 exposure. In this study, anti-IL-17C mAb attenuated inflammation in two distinct mouse models of AD and in the IL-23-induced psoriasis model. It is revealing that although IL-23 injection induces psoriasis, the inflammatory gene expression in the skin of treated mice is similar to that described for AD patients (Ewald et al., 2017). This reinforces the potential roles for IL-17C in psoriasis as well as AD.

In AD patients, recent Phase 3 data of dupilumab clinically underscore the important role of Th2 cells in disease pathogenesis, because IL-4Ra blockade, which affects IL-4 and IL-13 signaling, shows remarkable efficacy (Simpson et al., 2016). In the flaky tail model, the observed reductions of CCL17 level, IL-4 level, the number of mast cells, and IgE level upon IL-17C neutralization indicate that IL-17C contributes to an already established atopic inflammatory process, reinforcing the notion that in a clinical context, IL-17C is involved in disease maintenance and also acts beyond the initiation of disease.

In conclusion, IL-17C represents a target for treatment of inflammatory skin diseases. Supported by these findings, MOR106 is currently being evaluated in a phase 1 study in healthy volunteers and patients with moderate to severe AD to assess safety, tolerability, and pharmacokinetics of this IL-17C neutralizing antibody (NCT02739009).

**MATERIALS AND METHODS**

**Mice**

BALB/c mice (female, 8 weeks old) were obtained from Janvier Labs (Le Genest-Saint-Isle, France). Flaky tail (Matt ma/maFlgft/ft) mice were

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**Figure 3.** IL-17C neutralization attenuates the development of IL-23–induced psoriatic-like skin inflammation. Closed symbols and bars indicate IL-23 injection, and open symbols and bars indicate sham injection with PBS/BSA. (a) Time course of IL-23–induced ear swelling responses (mean ± standard deviation) in animals treated with MOR106 (n = 10) or with isotype antibody (n = 10) (both 10 mg/kg). Control animals (n = 5) receiving daily ear injections of PBS/BSA instead of IL-23 were treated with 10 mg/kg isotype antibody. (b) Histological assessment of epidermal and dermal thickness (in mm) mean measurements (± standard deviation) of ears sampled at day 5. (c) Mean relative mRNA expression levels (± standard deviation) of IL-17A, IL-22, IL-1β, LCN2, and S100A8/9 normalized to housekeeping genes in ears at day 5. Figures show data collected from a representative animal study. Studies have been repeated three times, and similar results were obtained for all readouts. **P < 0.01, ***P < 0.001. BSA, bovine serum albumin; D. Th., dermal thickness; Ep. Th., epidermal thickness; PBS, phosphate buffered saline.
Figure 4. IL-17C neutralization attenuates MC903-induced cutaneous inflammation and AD-like syndrome. Closed symbols and bars indicate topical application of MC903, open symbols and bars indicate topical application of ethanol (i.e., MC903 vehicle). (a) Time course of MC903-induced ear swelling responses (mean ± standard deviation) in animals treated with isotype control antibody (10 mg/kg), indicated doses of MOR106, or dexamethasone (DEX) (n = 8 each). (b) In vivo imaging of inflammation at day 5 using a cathepsin-sensitive probe (mean signal intensity, n = 8 each). (c) Histological assessment of epidermal and dermal thickness (in mm) measurements (mean ± standard deviation) of ears sampled at day 8 (n = 8 each). (d) CCL17 plasma levels and (e) TSLP and IL-33 protein in ear samples at day 8 (mean ± standard deviation) of ears sampled at day 8 (n = 8 each). (f) Microarray analysis of RNA from ears collected at day 8 each. Data in a–e for MOR106 at doses of 10 and 50 mg/kg have been repeated in another independent study, and similar results were observed. *P < 0.05, **P < 0.01, ***P < 0.001. AD, atopic dermatitis; D. Th., dermal thickness; DEX, dexamethasone; Ep. Th., epidermal thickness; EtOH, ethanol; Th, T helper.

backcrossed onto a C57BL/6j strain background, as described (Fallon et al., 2009; Saunders et al., 2013). Experiments were performed according to ethical guidelines applied by the Animal Institutional Care and Use Committee of Galapagos controlled by French authorities or in compliance with the Irish Department of Health and Children regulations and approved by the Trinity College Dublin’s BioResources ethical review board.

Expression analysis of IL-17C in human and mouse skin
Skin punch biopsy samples (6 mm) were obtained from healthy subjects and from lesional and clinically noninvolved skin from patients with established plaque psoriasis and AD. Written informed consent was obtained from all patients under protocols approved by the local ethical committee of the Technical University of Munich. Immunohistochemical stainings for human and mouse IL-17C expression were performed, respectively, on 4-μm paraffin sections with biotinylated anti-IL-17C antibody MOR22420 or MOR12743 and matching isotype control antibody (MorphoSys, Planegg, Germany) and detected by incubation with Avidin Biotin Peroxidase Complex (Vector Laboratories, Burlingame, CA) and peroxidase substrate diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St. Louis, MO). Mayer’s hematoxylin (BDH Chemicals, Radnor, PA) was used for counterstaining
before dehydration and mounting of slides. For qPCR, IL-17C expression was normalized to expression of 18S ribosomal RNA.

ELISA cross-reactivity with other IL-17 family members
MOR106 was tested for binding to human and mouse IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F (eBioscience San Diego, CA; Peprotech, Rocky Hill, NJ; Novus, St. Charles, MO; R&D Systems, Minneapolis, MN; BioLegend, San Diego, CA) by ELISA. Antigen-specific antibodies served as positive controls (eBioscience, R&D Systems).

Blocking of IL-17C binding to IL-17RE
Antibodies were incubated with equal volume of biotinylated IL-17C (mouse or human) for 30 minutes at room temperature, and after incubation, the level of free biotinylated IL-17C still able to bind to plates coated with human IL-17RE/Fc chimeric protein (Galapagos, Mechelen, Belgium) was detected via streptavidin-electrochemiluminescence using an MSD Sector Imager (Meso Scale Discovery, Rockville, MD).

IL-17C–driven NF-κB reporter assay
NIH3T3 mouse fibroblasts endogenously expressing mIL-17RA (but not mIL-17RE) were transfected (PolyPlus jet-PEI; Polyplus-transfection SA, Illkirch, France) with a mouse IL-17RE expression construct and a NF-κB luciferase reporter. Next, 10 μl of a serial antibody dilution that was pre-incubated for 30 minutes at room temperature with an equal volume of purified recombinant IL-17C (0.5 ng/ml; mouse, R&D Systems or human, Novus) was added to the wells. Luciferase was quantified after addition of SteadyLite Plus (PerkinElmer, Waltham, MA) on an EnVision reader (PerkinElmer).

IL-17C–driven gene expression in primary keratinocytes
Normal human primary keratinocytes were obtained from Lonza (Basel, Switzerland), and mouse primary keratinocytes derived from C57BL/6 mice were obtained from CELLnTEC (Bern, Switzerland). Cells were stimulated with 10 ng/ml mouse or human recombinant IL-17C (pre-incubated with or without different concentrations of antibody) in the presence of 10 ng/ml TNF-α (mouse or human, both from Peprotech) for 48 hours. The expression of DEFB4A (human) or CSF3 (mouse) was determined by qPCR.

IL-23–induced psoriasis model
The IL-23 psoriasis model was essentially carried out as described (Rizzo et al., 2011). In brief, skin lesions were induced in BALB/c mice by intradermal injection of murine IL-23 (1 μg) into the left ear for 4 consecutive days. Mice were killed on day 5. MOR106
(anti-IL-17C hulgG1) and MOR03207 (hulgG1 isotype control) were administered intraperitoneally (n = 10/group) 3 days before and just before the first injection of IL-23. Ear thickness was measured daily with an electronic caliper (Mitutoyo, Aurora, IL).

MC903 mouse model of AD
AD-like skin inflammation was induced in mice by MC903 as previously described (Li et al., 2006, 2009). Skin lesions were induced in BALB/c mice by topical administration of 2 nmol MC903 (Tocris, Bristol, UK; dissolved in ethanol) on both shaved ears for 5 consecutive days. Mice were killed on day 8. Antibodies were administered intraperitoneally (n = 8/group) 3 days before, at the start, and 4 days after the first application of MC903. One group treated with dexamethasone (5 mg/kg orally, every day starting on the first day of MC903 application) served as a steroid comparator. Ear thickness was measured daily with an electronic caliper (Mitutoyo). Ear inflammation was assessed on day 5 by in vivo molecular imaging, 24 hours after injection of a cathepsin-sensitive Prosense 680 probe (0.8 nmol/10 g, intraperitoneally) (PerkinElmer). All images were captured using the Bruker In-vivo Xtreme Imaging System (Bruker, Billerica, MA).

Flaky tail model
Flaky tail (Mattnma/maFlgft/ft) mice on a congenic C57BL/6J background were used as described (Fallon et al., 2009; Saunders et al., 2013). Female flaky tail mice of 9–10 weeks old were randomized into four groups (n = 8/group) and treated for 6 weeks with antibodies given intraperitoneally twice weekly. One group treated with dexamethasone (2 mg/kg intraperitoneally, twice weekly) served as a steroid treatment comparator. The severity of AD-like skin inflammation was scored using the macroscopic diagnostic criteria as described (Saunders et al., 2013).

Histomorphometric analysis of mouse skin
Skin samples were fixed in 4% formaldehyde before embedding in paraffin. Next, 4-μm-thick sections of ears were stained with hematoxylin and eosin. Epidermal thickness (acanthosis) was evaluated using image analysis. Immune cell quantification in the skin was performed as previously described (Ward et al., 2011).

RNA isolation and quantitative real-time PCR
Mouse skin samples were submerged in RNAlater stabilization solution (Ambion, Naugatuck, CT) and stored at 4°C. Tissue was disrupted in Trizol (ThermoFisher Scientific Waltham, MA) using a Precellys homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France). Total RNA was extracted using the NucléoSpin RNA Kit (Macherey-Nagel, Düren, Germany). For qPCR, RNA was reverse-transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). qPCR was performed on the ViiA 7 Real-Time PCR System (Applied Biosystems) using SYBR green technology with gene-specific primers from Qiagen (Hilden, Germany).

Microarray gene expression analysis
Microarray gene expression analysis was performed using Agilent (Santa Clara, CA) SurePrint G3 mouse chips. Quality control of microchips was carried out using the Bioconductor package array QualityMetrics (Huber et al., 2015). Background correction and across array normalization was performed using limma (R, Bioconductor). Differential expression between groups was determined using general linear models and empirical Bayes moderated t statistics; multiple testing P-value correction was applied (false discovery rate method; all analyses were performed in limma).

Skin protein measurements
Ear skin samples were snap frozen in liquid N2 and stored at -80°C. Tissues were disrupted in lysis buffer (T-PER Tissue Protein Extraction Reagent, Pierce, Puyallup, WA) supplemented with Protease Inhibitor Cocktail (Roche, Basel, Switzerland) and Halt Phosphatase Inhibitor Cocktail (Pierce) using a Precellys homogenizer. Cytokines of interest were determined by ELISA, and the amount was normalized to total protein content.

ELISA
ELISAs were performed to quantify specific cytokines in serum (IL-17A, CCL17, IFN-γ; R&D Systems; IL-4: PharMingen, San Diego, CA). Total IgE was measured as described (Fallon et al., 2009) using PharMingen antibodies. TSLP and IL-33 were measured in ear skin samples using a standard ELISA (R&D Systems).

Statistical analysis
Data are presented as mean ± standard deviation. Statistical analysis and comparison of treatment groups was performed with a one-way analysis of variance and Dunnett post hoc test. For qPCR data, statistical analyses were run on Rq values. P-values less than 0.05 were considered significant.

CONFLICT OF INTEREST
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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.01.036.

REFERENCES


