Dysregulated skin barrier function in Tmem79 mutant mice promotes IL-17A-dependent spontaneous skin and lung inflammation

Sean P. Saunders1 | Achilleas Floudas1 | Tara Moran1,2 | Ciara M. Byrne1 | Michael D. Rooney1 | Caoimhe M. R. Fahy1,3 | Joan A. Geoghegan4 | Yoichiro Iwakura5 | Padraic G. Fallon1,2 | Christian Schwartz1,6

1Trinity Biomedical Sciences Institute, School of Medicine, Trinity College Dublin, Dublin, Ireland
2National Children’s Research Centre, Our Lady’s Children’s Hospital, Crumlin, Dublin, Ireland
3Dermatology, Our Lady’s Children’s Hospital Crumlin, Dublin, Ireland
4Department of Microbiology, Moyne Institute of Preventive Medicine, School of Genetics and Microbiology, Trinity College Dublin, Dublin, Ireland
5Research Institute for Biomedical Sciences, Tokyo University of Science, Yamazaki, Japan
6Mikrobiologisches Institut - Klinische Mikrobiologie, Immunologie und Hygiene, Universitätsklinikum Erlangen and Friedrich-Alexander Universität (FAU) Erlangen-Nürnberg, Erlangen, Germany

Abstract

Background: Atopic dermatitis (AD) is associated with a dysregulation of the skin barrier and may predispose to the development of secondary allergic conditions, such as asthma. Tmem79ma/ma mice harbor a mutation in the gene encoding Transmembrane Protein 79 (or Mattrin), which has previously been associated with AD. As a result of the Tmem79 gene mutation, these mice have a defective skin barrier and develop spontaneous skin inflammation. In this study, Tmem79ma/ma mice were assessed for the underlying immunological response in the development of spontaneous skin and lung inflammation.

Methods: Development of spontaneous skin and lung inflammation in Tmem79ma/ma mice was analyzed. We further investigated susceptibility to cutaneous Staphylococcus aureus infection. Tmem79ma/ma were crossed to IL-17A-deficient mice to address the contribution of IL-17A to spontaneous skin and lung disease.

Results: Tmem79ma/ma mice developed IL-17A-dependent spontaneous AD-like inflammation and were refractory to S aureus infection. Mutant mice progressed to airway inflammation subsequent to the occurrence of dermatitis. The progression from skin to lung disease is dependent on adaptive immunity and is facilitated by cutaneous expansion of Th17 and TCRγδ T cells.

Abbreviations: AD, atopic dermatitis; AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; IL, interleukin; ILC, innate lymphoid cell; SNP, single nucleotide polymorphism; WT, wild-type.

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INTRODUCTION

Atopic dermatitis (AD) is a common inflammatory skin disorder affecting 15%-30% of children in the western world. Individuals with a history of AD are predisposed to develop secondary allergic conditions in distal tissue sites, such as the lung, in what is known as the atopic march. The etiology of AD is multifactorial, with roles for genetic predisposition and environmental factors in the development of aberrant skin inflammation. Disease progression is complex and significantly influenced by the interplay between the functional integrity of the skin barrier and the immune system.

Recent studies have illustrated the complexity and heterogeneity of inflammatory gene expression in the skin of patients with AD. Therefore, the spectrum of underlying immune responses associated with AD now includes CD4+ T helper (Th)2, IL-22 (Th22), IL-17 (Th17) as well as IL-23 mediated cellular responses in different patient cohorts, and in mouse models that develop AD-like inflammation. There is increased expression of IL17A in both acute and chronic lesions of patients with AD.

While studies have indicated a potential role for IL17A in disease pathogenesis, the function and source of IL-17A in AD patients are not well defined. In mouse models of skin inflammation, IL-17A has been shown to be involved in the generation of AD-like skin inflammation, with a critical role for microbiota-induced IL-17A secretion.

The flaky tail double-mutant mouse, with mutations in Filaggrin (Flg) and Tmem79, has been extensively used for studies of skin barrier defects and development of AD-like skin inflammation. The skin inflammation in flaky tail mice on C57BL/6 background is due to mutations in Tmem79, and not Flg. Tmem79mutant mice do not express the transmembrane transport protein mattrin leading to a defective skin barrier and the development of skin inflammation. The identification of the Tmem79 gene in mice led to detection of a missense SNP in the human homologue, TMEM79/MATT, that significantly increases the risk for AD.

In this study, we now demonstrate that the Tmem79mutant mice develop spontaneous IL-17A-dependent skin inflammation with secondary progression to lung pathology. Indeed, cutaneous
expansion of IL-17A-expressing Th17 and TCRγδ cells facilitated progression to pulmonary inflammation, which provides important insights into the pathogenesis of the atopic march.

2 METHODS

2.1 Mice

The Tmem79ma mutation was separated from double-mutant flaky tail (Mattma/Flgft/ft) mice, and Tmem79ma mice were backcrossed to congenic C57BL/6J background as previously outlined.16 Homozygous female Tmem79ma/ma mice were used in all experiments. TCRδ-deficient (Strain No: 002120, Jackson Laboratories), TCRα-deficient (Strain No: 002116, Jackson Lab), Il17a-/− mice, and Rag1-deficient (Rag1−/− ; Strain No: 003145; Jackson Labs) mice, were crossed with Tmem79ma/ma mice in-house to generate dual TCRδ−/−Tmem79ma, TCRα−/−Tmem79ma, Il17a−/−Tmem79ma, IL-17eGFP/Tmem79ma/ma, and Rag1−/−Tmem79ma/ma mice, respectively. Conditional Tmem79 floxed mice were generated by introducing LoxP sites flanking exon 2 of Tmem79 (Ozgene), according to the targeting strategy described in Figure S2. Tmem79fl/fl mice were crossed to β-actin-cre (ACTB-cre; Strain No: 003376) and human keratin 14-cre strains (K14-cre; Strain No: 003145; Jackson Labs) in-house.

Mice were housed in specific pathogen-free conditions, with irradiated diet and bedding and water ad libitum. All animal care and experimental procedures were performed under an Irish Department of Health and Children licensed procedures (holder Padraic Fallon, Licence Number B100/3250) and Irish Health Products Regulatory Authority (Authorization Number: AE19136/P071). Animal experiments received ethical approval from the Trinity College Dublin Bioresources Ethical Review Board (Reference: 121108).

2.2 Clinical scoring

The severity of inflammation and AD-like pathology was scored using the macroscopic diagnostic criteria as described for the skin inflammation in the Nc/Nga mouse model of AD.19 A scoring system (score 0, none; score 1, mild; score 2, moderate; score 3, severe) was applied to the symptoms of edema, erosion, scaling, and erythema. Total scores for each mouse, to a maximum of 12, were calculated from the sum of individual parameters.

2.3 Scratching behavior

Individual mice were videoed and the spontaneous scratching of mice was quantified as described.20 Scratches per minute were determined based on an individual animal lifting a hind paw from the cage floor, scratching with the paw, and then returning the paw to the cage floor.

2.4 Trans-epidermal water loss (TEWL)

The measurement of TEWL was performed on ear skin using a Courage and Khazaka Tewameter TM210 (Envirotex), as described previously.15

2.5 Histology

Skin or lungs were fixed in 10% formaldehyde saline. Skin sections were stained with hematoxylin and eosin (H&E). Lung sections were stained with H&E and Masson’s trichrome for collagen quantification, as described.21

2.6 RNA isolation and real-time PCR

RNA was isolated from skin using the RNeasy kit and reverse transcribed with the Quantitect reverse transcription kit (Qiagen). Real-time quantitative PCR was performed on an AB StepOnePlus Real-time PCR system (Life Technologies), using TaqMan gene expression assays specific for murine Ifnγ (Mm01168134_m1), Il4 (Mm00445259_m1), Il13 (Mm00434204_m1), Il17a (Mm00439618_m1), Il1β (Mm00434228_m1), Il13 (Mm00505403_m1), Tslp (Mm01157588_m1), and Il25 (Mm00499822_m1). Specific gene expression was normalized to murine glyceraldehyde-3-phosphate dehydrogenase (VIC probe, 4352339E; Life Technologies).

2.7 Airway hyperresponsiveness analysis

Airway hyperresponsiveness (AHR) was analyzed in 32-week-old mice. Mice were tracheostomized and ventilated using a whole-body plethysmography, with a pneumotachograph linked to a transducer (EMMS). Changes in lung resistance (RL) and compliance (Cdyn) in response to increasing doses of nebulized and inhaled methacholine (10, 30, 60, and 100 mg/mL; Sigma-Aldrich), were recorded as described.15,21

2.8 Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) fluid was collected following AHR analyses.22 Differential cell counts were performed on BAL cells on cytopsins.

2.9 Collagen assay

Briefly, lung tissue was collected and homogenized, with subsequent overnight acid-pepsin collagen extraction at 4°C. Total soluble collagen content was determined with a Sircol Collagen Assay kit (Biocolor). Pulmonary collagen was expressed as μg collagen per mg lung protein.21
2.10 | Protein extraction from lung and cytokine measurement

Lungs were homogenized in a buffer containing PBS, 2% fetal bovine serum and 0.5% cetyltrimethylammonium bromide using an IKA T10 Basic Ultra-Turrax homogenizer. The homogenates were centrifuged at 13,000 g for 15 minutes. Cytokines were determined using commercial ELISA kits (R&D systems) and expressed as ng cytokine per mg lung protein.

2.11 | Staphylococcus aureus infections

Mice were infected with a bioluminescent S aureus strain Xen 36 (Caliper Life Sciences Inc) derived from S aureus ATCC 49525. The dorsal skin of mice was shaved 24 hours prior to intradermal injection of 2 x 10^5 bacteria. Skin lesion size was measured daily for 10 days. Staphylococcus aureus bioluminescence at the infection site was quantified daily using an IVIS Lumina Series III Pre-Clinical In Vivo imaging system (IVIS 100, Caliper Life Sciences). Bioluminescence activity, measured as photons/sec/cm^2/steradian, was quantified using Living Image Software (V. 3.0.2; Caliper LS) with the infection site as the designated region of interest (ROI). After 10 days, the infection site was removed and either fixed for histology or homogenized and analysis of bacterial counts in skin sites were determined.

2.12 | Cell preparation and flow cytometric analyses of skin-draining lymph node and ear or lung tissue

Skin-draining lymph node cells were isolated for flow cytometric analyses. Skin was excised and incubated overnight at 4°C in 3 mg/ml Dispase I (Sigma) in Hank’s Buffered Salt Solution (Sigma). Skin was removed from dispase and incubated for 1 hour at 37°C in an orbital incubator with 1 mg/mL Collagenase D (Roche) in PBS. Lung tissue was minced and incubated for 30 minutes at 37°C in an orbital incubator with 1 mg/mL Collagenase D (Roche) in PBS. For surface staining, single-cell suspensions were prepared in flow-cytometry buffer (PBS, 2% FBS, 0.05% sodium azide). LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Life Technologies, UK) was used to determine cell viability. Cells were stained with all eBioscience unless indicated otherwise (CD4 (RM4-5), CD8 (53-6.7), CD11b (M170), CD11c (HL3), CD19 (ID3), CD45.2 (104), FcγRI (MAR-1), F4/80 (BM8), Gr-1 (RB6-8C5), I-A/I-E (2G9), ICOS (7E.17G9), IL-7R (HL3), Sca-1 (D7), TCR-β (H57-597), Ter119 (TER119), CD3 (17A2), Ly6G (1A8; Biolegend), TCR γδ (GL3; Biolegend), and T1/ST2-FITC (DJ8; MD biosciences). For intranuclear transcription factor expression, cells were stained with BD Biosciences mAbs; Tbet (O4-46), RORγT (Q31-378) and GATA3 (L50-823) following fixation and permeabilization with the FOXP3 staining kit (eBioscience). Cells were acquired using a Cyan (ADP Analyzer; Beckman Coulter) and analyses were performed using FlowJo software (Tree Star Inc).

2.13 | Cell stimulation

Skin-draining lymph nodes cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% heat-inactivated FBS (Sigma-Aldrich), 2 mmol L-glutamine (Invitrogen), 50 IU/mL penicillin, and 50 μg/mL streptomycin (Invitrogen). Cells were stimulated in vitro with plate-bound anti-CD3 (10 μg/mL; 2C11, BD) and anti-CD28 mAb (2 μg/mL; 37.51, BD) or 25 ng/mL phorbol 12-myristate 13-acetate (PMA) and 250 ng/mL ionomycin for 72 hours, after which supernatants were collected.

2.14 | IgE and cytokine ELISA

Total serum IgE was measured in serum from mice according to the manufacturer’s instructions (BD Pharmingen). Cytokines in cell culture supernatants were analyzed by ELISA as per the manufacturer’s instructions (R&D Systems).

2.15 | Statistical analyses

Data are expressed as mean ± SEM and were analyzed by two-way analysis of variance (ANOVA) test or unpaired Student’s t tests (Prism 7; GraphPad Software). Significance for was shown in figures as: NS = not significant, P < .05 (*), P < .01 (**), P < .001 (***), and P < .0001 (****).

3 | RESULTS

3.1 | Tmem79-deficiency leads to spontaneous skin inflammation

Tmem79^ma/ma mice spontaneously develop skin inflammation in adulthood, progressing from 8 weeks of age to overt dermatitic skin inflammation in all mice, characterized by edema, erythema, scaling and thickening in multiple skin sites, with excoriation apparent in the ventral flank by 32 weeks of age (Figure 1A, B). This reinforces the specific role for the mutation in Tmem79 in the genesis of spontaneous skin inflammation in flaky tail strain mice - with Flg^ft/ft mutant mice on C57BL/6 background developing no skin inflammation (Figure. S1A). There is profound acanthosis in Tmem79^ma/ma mice with inflammatory cell infiltration into the dermis including eosinophils and neutrophils in lesional skin (Figure 1C). Furthermore, Tmem79^ma/ma mice showed signs of pruritis evidenced by a significant increase in scratching behavior (Figure 1D). Expression analysis for a panel of genes implicated in cutaneous inflammation in lesional skin of Tmem79^ma/ma mice revealed statistically significant elevation of
Il17a (P < .01), Ifng (P < .05), Il1b (P < .05) and Il13 (P < .05) transcripts relative to WT control mice (Figure 1E). In nonlesional skin sites of Tmem79ma/ma mice, there was a specific elevation in Il17a (P < .01), but not expression of the other cytokines, indicating a type 17 cutaneous cytokine milieu in the noninflamed skin of Tmem79 mutant mice (Figure 1E and Figure S1B). These data show that Tmem79ma/ma mice spontaneously develop marked dermatitis, which was associated with increased Il17a expression in the skin that was present prior to the development of overt skin inflammation.

In patients with AD, infection with S aureus is implicated in disease pathogenesis.23,24 To address the effects of Tmem79ma/ma mutation on S aureus skin infection, mutant and WT mice were infected with a bioluminescent strain of S aureus. Mutant mice had significantly impaired S aureus colonization of the skin relative to WT mice (Figure 2A,B), with significantly reduced bacteria detected at the site of infection (Figure 2C). The diminished colonization of skin was reflected by reduced development of an inflammatory lesion at the site of infection in mutant mice (Figure 2D,E). Cells from the skin-draining LN at the site of infection were isolated, restimulated in vitro, and production of cytokines associated with Th1 (IFN-γ), Th2 (IL-4) and Th17 (IL-17A) was analyzed. Notably, LN cells, in particular in vitro activated T cells, from Tmem79ma/ma mice already produce more IL-17A and IFN-γ under noninfected conditions (Figure 2F), which reflects the ongoing skin inflammation. However, upon infection with S aureus, LN cells from infected mutant mice produced significantly (P < .001) more IL-17A than WT mice (Figure 2F). While production of IFN-γ and IL-4 was also increased by LN cells from Tmem79ma/ma mice, the increase was not statistically compared to cells from infected WT animals (Figure 2F). These results demonstrate that spontaneous skin inflammation in Tmem79ma/ma mice renders them refractory to S aureus infection and was characterized by an IL-17A-dominant cutaneous immune response.

### 3.2 | Tmem79ma/ma mice develop spontaneous pulmonary inflammation

As AD can initiate the progression to allergic pulmonary inflammation, we analyzed the lungs of Tmem79ma/ma mice. At 16 weeks of age, mutant mice had developed skin inflammation (Figure 1A) but there was no overt lung inflammation (Figure S1C). However, subsequent analysis at 32 weeks of age revealed that Tmem79ma/ma mice had alterations in AHR, with significant changes in dynamic lung compliance (C_{dyn}) in mutant mice compared to WT controls but not in lung resistance (R_L) (Figure 3A).

In support of the altered lung function and presence of pulmonary inflammation, there were significantly (P < .05) more cells in BAL of Tmem79ma/ma mice relative to WT mice with an increase in total cell numbers observed across all cell populations (Figure 3B). Notably, there was a significant increase in the frequency of both neutrophils...
The compromised AHR in Tmem79ma/ma mice was reflected in marked lung pathology with mixed peribronchial cellular infiltrates (Figure 3C). The lungs of Tmem79ma/ma mice did not develop bronchial goblet cell hyperplasia, marked peribronchial eosinophilia, or airway occlusion (data not shown). In lung homogenates from Tmem79ma/ma mice, IL-17A was significantly ($P < .0001$) increased, as was IL-1β ($P < .0001$; Figure 3D). The alarmins IL-25 ($P < .01$) and TSLP ($P < .05$) were also significantly elevated in the lungs of Tmem79ma/ma mice (Figure 3D), whereas IL-33 was unchanged (Figure 3D). Levels of IL-4, IL-13, IFN-γ, and TGF-β were comparable in the lungs of WT and Tmem79ma/ma mice (Figure 3D). Consistent with the altered peripheral pulmonary changes in the lung, Tmem79ma/ma mice had marked collagen deposition (Figure 3E, F).

In humans, TMEM79/mattrin is expressed in a restricted number of tissues with high expression in the skin, prostate, tongue, and cervix. Tmem79ma/ma mice have therefore ablated expression of mattrin in multiple organs. This raises the question if expression in the skin is central to defects in the skin barrier leading to the genesis of inflammation and subsequent pulmonary pathology. To address tissue-specific functions of Tmem79, we generated Tmem79fl/fl mice (Figure S2A). Tmem79fl/fl mice were crossed to ACTB-cre mice, with ubiquitous Cre recombinase expression controlled by the β-actin promoter in all cells, for a total excision of Tmem79. Tmem79fl/fl ACTB-cre mice developed a phenocopy of constitutive Tmem79 mutant mice, confirming the efficient deletion of Tmem79 by Cre-lox-mediated recombination (Figure S2B). In order to specifically delete Tmem79 in keratinocytes, we generated Tmem79fl/flK14-cre mice (Figure S2A). These mice with ablation of cutaneous mattrin expression had a defective skin barrier, developed spontaneous, pruritic, and progressive skin and lung inflammation,

Figure 2: Tmem79-deficient mice are refractory to skin S aureus infection. A, IVIS imaging of Tmem79ma/ma vs wild-type (WT) mice over 10 d following infection of dorsal skin by intradermal injection of $2 \times 10^5$ bioluminescent $S$ aureus. B, Quantification of bioluminescence at infection site. C, Quantification and images of bioluminescent $S$ aureus cultured from the infection site of WT and Tmem79ma/ma mice. D, Gross appearance of infection site of mutant and WT mice after 5 d. E, Skin lesion area at infection site of WT and Tmem79ma/ma mice at indicated time points after infection. F, Quantification of IL-17A, IFN-γ and IL-4 produced by cells isolated from skin-draining lymph nodes following in vitro activation with PMA/ionomycin or anti-CD3 and anti-CD28 mAb. *$P < .05$; **$P < .001$. Student’s t test
a phenocopy of the mutant mouse and disease progression in mice with ubiquitous β-actin-cre-mediated conditional deletion of matrin (Figure S2A-C). This highlights that matrin expression in the skin has a role in maintaining skin homeostasis and mice with keratinocyte-specific deficiency in matrin, develop a compromised skin barrier that results in cutaneous and pulmonary inflammation (Figure S2D).

Collectively, Tmem79ma/ma mice spontaneuously develop lung pathology, that was secondary to skin inflammation, with decreased lung compliance, increased parenchymal collagen deposition, eosinophil and neutrophil infiltration, and pulmonary inflammation associated with IL-17A.

3.3 | Tmem79ma/ma mice have an expansion of IL-17A producing T cells

Given the pronounced increase in IL-17A levels in both lesional and nonlesional skin of Tmem79ma/ma mice, we investigated the cellular source of IL-17A by generating dual Il17eGFP reporter Tmem79ma/ma mice. Assessing the levels of IL-17A-producing T cells in the skin-draining LN of Tmem79ma/ma mice, prior to the development of eczematous skin inflammation, revealed that IL-17A was expressed by both γδ T cells and CD4+ Th cells (Figure 4A,B). In Tmem79ma/ma mice that develop lesional inflammation, analyses of the levels of Th1 (TBET+CD4+), Th2 (GATA3+CD4+) and Th17 (RORγT+CD4+) cells, revealed specific expansion of Th17 cells in skin-draining LN (Figure S3A). In the lesional skin itself, a significant expansion of IL-17γδ T cells and IL-17+ CD4+ T cells was observed (Figure 4C, D). We also assessed IL-17A production by type 3 innate lymphoid cells (ILC3), an innate producer of IL-17A that has been associated with skin inflammation.25-27 However, the frequency of both ILC3 (Lin-IL-7Rα+ST2+IL-17eGFP+) and ILC2 (Lin-IL-7Rα+ST2+) was comparable in mutant and wild-type mice (Figure S3B).

The lung inflammation that develops in Tmem79ma/ma mice was associated with increased IL-17A levels in lung homogenates (Figure 3D), yet Il17eGFP Tmem79ma/ma mice experienced no
significant increase in the frequency of pulmonary IL-17+ γδ T cells or Th17 cells (Figure 4E,F). Similar to the skin, the lungs of mutant mice had comparable frequencies of ILC2, ILC3 and Th2 cells to wild-type mice (Figure S3C; data not shown). Taken together these data indicate that skin inflammation in Tmem79ma/ma mice is associated with a type 17 cellular response.

3.4 | Development of spontaneous skin and lung inflammation in Tmem79ga/ma is mediated by adaptive immunity

We sought to address if inflammation in Tmem79ga/ma mice was mediated by adaptive immunity by generating T and B cell deficient Rag1−/− Tmem79ga/ma mice and, since γδ T cells are increased in skin of mutant mice, dual Tcrδ−/−Tmem79ga/ma mice (Figure 5A). Both Rag1−/−Tmem79ga/ma and Tcrδ−/−Tmem79ga/ma mice were protected from overt skin inflammation (Figure 5A). In contrast, dual Tcrα−/−Tmem79ga/ma mice developed skin inflammation (data not shown). The defect in the skin barrier of Tmem79ga/ma mice, evident by significantly elevated (P < .005) TEWL in nonlesional skin of 8-week-old mutant mice compared to WT mice, was also present in the dual Rag1−/− or Tcrδ−/−Tmem79ga/ma mice (Figure 5B). Importantly, both strains of mice did not progress to develop lung pathology (Figure 5C,D), indicating a critical role for T and B cells, and among these specifically γδ T cells, in the pathogenesis of dermatitis and progression to lung pathology in Tmem79ga/ma mice. Our data suggest that development of spontaneous skin and lung inflammation in Tmem79ga/ma mice are dependent on TCR γδ T cells.

3.5 | Spontaneous skin and lung inflammation in Tmem79ga/ma mice is dependent on IL-17A

In order to define the contribution of IL-17 to inflammation, II17a−/−Tmem79ga/ma mice were generated. II17a−/−Tmem79ga/ma mice had a profound amelioration in the magnitude of skin inflammation (Figure 6A,B), with significantly (P < .0001) reduced clinical skin inflammation relative to Tmem79ga/ma controls (Figure 6A). Consequently, scratching was significantly reduced in IL-17A-deficient Tmem79ga/ma mice (Figure 6B). Overt inflammatory lesions did not develop and dermal inflammatory cell infiltration and epidermal hyperplasia were decreased in II17a−/− Tmem79ga/ma mice.
At 32 weeks of age, IL-17A-deficient mutant mice had no lung inflammation, while Tmem79<sup>ma/ma</sup> mice had developed pronounced lung pathology (Figure 6D,E). These results demonstrate that pathogenesis of overt skin inflammation as well as the progression to lung pathology in mice with an impaired skin barrier due to Tmem79-deficiency is dependent on IL-17A.
Increasing evidence from genome-wide association studies (GWAS), immunochip and transcriptome analyses firmly implicate genetic predisposition associated with skin barrier defects, as a causal factor in AD pathogenesis. Previous studies on a mouse model with a mutation in Tmem79, identified in the Flaky tail dual Flg-Tmem79 mutant strain, with an impaired skin barrier, led to the demonstration of a missense SNP in Tmem79 that was associated with human AD. In contrast, mice with a mutation in the filaggrin gene (Flg<sup>ft/ft</sup>) develop no overt skin inflammation on the C57BL/6 background (Figure S1A), while they develop AD-like inflammation on the BALB/c background in an IL-1β- and mast cell-dependent manner. We now show that Tmem79<sup>ma/ma</sup> mice develop spontaneous skin inflammation that progresses to lung inflammation, through an IL-17A dependent mechanism that is mediated by TCRγδ T cells.

The AD-like skin inflammation in Tmem79<sup>ma/ma</sup> mice is characterized by a ~40-fold increase in IL17A expression, and a marked infiltration of both Th17 and IL-17+ γδ T cells. TCRγδ T cells are major producers of IL-17A and rapidly respond to dysregulation of the skin barrier. Importantly, we observe a specific ~7-fold increase in IL17A in nonlesional skin, which is associated with an increase in the frequency of skin IL-17+ γδ T cells in mutant mice prior to overt skin inflammation. These high basal levels of IL-17A in the skin of Tmem79<sup>ma/ma</sup> mice mediate refractoriness to S aureus challenge. Indeed, specific alterations in responses toward S aureus have been reported in a number of other mouse strains with defective skin barrier integrity. The upregulation in Il1β expression in the nonlesional skin of both Tmem79<sup>ma/ma</sup> mice is consistent with previous work, in which epidermal IL-1β was shown to be important in AD patients and murine AD models. As IL-1β can activate IL-17+γδ T cells in the skin, the increased IL-1β in skin of mutant mice may increase the frequency of cutaneous IL-17+γδ T cells to amplify skin inflammation suggesting that IL-1β acts upstream of IL-17A-mediated pathology. Indeed, in other mouse models of disrupted skin barrier, there is increased infiltration of IL-17+γδ T cells in the skin in two distinct mouse models of defective epidermal barrier leading to cutaneous inflammation, γδ T cells were increased in the skin but were redundant for the genesis of skin inflammation. In Tmem79<sup>ma/ma</sup> mice the development of spontaneous IL-17A-dependent skin inflammation was impaired in the absence of T and B cells as well as TCRδ cells, but not TCRα cells. Although we currently cannot exclude a role for Th17 cells, which are also increased in lesional skin, the absence of frank skin and lung inflammation in both Rag1<sup>+/−</sup>Tmem79<sup>ma/ma</sup> and Tcrδ<sup>−/−</sup>Tmem79<sup>ma/ma</sup> mice suggests a pathogenic function of IL-17-expressing TCRγδ cells. At the same time, both Rag1<sup>+/−</sup> and Tcrδ<sup>−/−</sup> mutant strains had defects in their skin barriers comparable to Tmem79<sup>ma/ma</sup> mice, indicating that T cells and in particular, TCRγδ T cells are essential to initiate skin inflammation in Tmem79<sup>ma/ma</sup> mice with an underlying impaired skin barrier.

We generated dual IL-17A-deficient and Tmem79<sup>ma/ma</sup> mice that demonstrated that the development of skin and lung inflammation in mutant mice was IL-17A-dependent. Previously, roles for IL-17A in skin inflammation have been reported in the Flaky tail mice that the Tmem79 mutant mice were derived from. While one study suggested that IL-17A-mediated inflammation was a consequence of the filaggrin mutation present in flaky tail mice, we could now show that in fact the Tmem79<sup>ma/ma</sup> mutation was responsible for increased IL-17A production and neutrophil infiltration. In mice with defective IL-17A signaling the skin barrier is compromised, with IL-17A treatment of primary murine keratinocytes altering filaggrin monomer formation. IL-17A treatment of human keratinocytes has been shown to downregulate monomeric filaggrin protein, and the expression of genes implicated in keratinocyte adhesion. Therefore, mutations in Tmem79 may further decrease skin barrier integrity through modulating filaggrin expression by increased IL-17A production. These studies highlight that IL-17A has a central role in skin barrier maintenance and the development of cutaneous inflammation in Tmem79<sup>ma/ma</sup> mice. Future studies are needed to address the interplay of mutations affecting skin barrier integrity and the ensuing AD to personalize therapy in affected AD patients.

The pulmonary inflammation in Tmem79<sup>ma/ma</sup> mice was characterized by increased collagen deposition, eosinophil and neutrophil infiltration in the airways, and increased IL-17A and IL-1β, leading to compromised lung function. While Tmem79<sup>ma/ma</sup> mice have altered dynamic lung compliance, there is no change in airway resistance, a surrogate marker for central airway allergic inflammation. These alterations in lung compliance in Tmem79<sup>ma/ma</sup> mice suggest that the aberrant lung function is predominately due to peripheral changes, such as lung parenchyma elasticity, with lesser effects on central airway function. In mouse models, it has been previously shown that Th17 cells mediate steroid-resistant airway inflammation and are capable of inducing AHR and neutrophil recruitment in response to Ovalbumin-challenge. Indeed, an IL-17A(high) immune phenotype has been described in patients with steroid-resistant asthma. We herein present the first example of a mouse model of IL-17A-dependent spontaneous lung inflammation that occurs subsequent to spontaneous skin inflammation. Currently, the main source of the increased IL-17A in the lung remains unclear. While we observed a minor increase in the frequency of pulmonary TCRγδ and Th17 cells, their increased cell numbers most likely increase the overall abundance of IL-17A. Furthermore, several other cells have been reported to produce IL-17A, including neutrophils and eosinophils.

It will be interesting to further explore the sensitivity of Tmem79<sup>ma/ma</sup> mice to steroids, or other interventions, to treat pulmonary inflammation. The exact factors that drive the atopy march are still not entirely clear. Thus, both Tmem79<sup>ma/ma</sup> and Flg<sup>ft/ft</sup> mice, which develop pulmonary inflammation following overt AD-like inflammation, are valuable tools to investigate the factors promoting allergic escalation.
We generated Tmem79fl/fl mice to conditionally delete mattrin in a cell- or site-specific manner. Total deletion of Tmem79, using ACTB-Cre mice, validated the Cre-lox system with mice developing comparable phenotypes as the mutant strain. Conditional targeting using K14-cre mice, demonstrated that keratinocyte-derived Tmem79 was responsible for the spontaneous skin and lung inflammation observed in Tmem79ma/ma mice. Indeed, others have generated a similar mouse model and could show that deletion of Tmem79 in keratinocytes increased expansion of IL-17A+ γδ-T cells and elicited mast cell-mediated histaminergic itch.44 While they did not observe overt lung inflammation, we only observed pulmonary inflammation starting after 16 weeks of age (Figure S1), which may be explained by the different age of mice used in both studies. The Tmem79fl/fl mouse model will serve as novel resource for studies on the functions of Tmem79 in the skin as well as other conditions, such as neurologic stimulation44 and prostate cancer.45 That Tmem79 is implicated. Future studies will employ the Tmem79ma/ma mouse model of skin barrier defect-driven AD to further delineate the function of IL-17A in the progression of cutaneous inflammation to leading secondary lung inflammation.

ACKNOWLEDGMENTS
We are grateful to Alan Irvine, Irwin McLean, and Tim Foster for assistance. PGF is supported by the Irish Research Council, the National Children’s Research Centre, Science Foundation Ireland (10/I1/1/B3004), and the Wellcome Trust (092530/Z/10/Z). CS is supported by the Interdisciplinary Center for Clinical Research (i2KF) at the University Hospital of the University Erlangen-Nuremberg (J79), EMBO (ALTF 587-2016), and the Else Kröner-Fresenius-Stiftung (2019_A181). Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST
Dr Saunders has nothing to disclose. Dr Floudas has nothing to disclose. Dr Moran has nothing to disclose. Dr Byrne has nothing to disclose. Dr Rooney has nothing to disclose. Dr Fahy has nothing to disclose. Dr Geoghegan has nothing to disclose. Dr Iwakura has nothing to disclose. Dr Fallon has nothing to disclose. Dr Schwartz has nothing to disclose.

AUTHOR CONTRIBUTIONS
SPS designed, performed and analyzed experiments and wrote the draft manuscript. AF, TM, CMB, MDR, and CMRF performed or contributed to specific experiments. JG and YI provided essential reagents. PGF and CS conceptualized the study, designed and supervised the experiments, analyzed data, and wrote the manuscript.

ORCID
Sean P. Saunders  doi: https://orcid.org/0000-0003-1689-3598
Achilleas Floudas  doi: https://orcid.org/0000-0003-1690-5595
Yoichiro Iwakura  doi: https://orcid.org/0000-0002-9934-5775
Padraic G. Fallon  doi: https://orcid.org/0000-0002-8401-7293
Christian Schwartz  doi: https://orcid.org/0000-0002-7084-268X

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Saunders SP, Floudas A, Moran T, et al. Dysregulated skin barrier function in Tmem79 mutant mice promotes IL-17A-dependent spontaneous skin and lung inflammation. Allergy. 2020;75:3216–3227. https://doi.org/10.1111/all.14488