Imiquimod-Induced Psoriasis in Mice Depends on the IL-17 Signaling of Keratinocytes
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The pathology of psoriasis strongly depends on IL-17A. Monoclonal antibodies blocking either the cytokine or its receptor are among the most efficient treatments for psoriatic patients. Keratinocytes can be activated upon exposure to IL-17A and tumor necrosis factor-α and secrete secondary cytokines and chemokines in the inflamed skin. In psoriasis and its imiquimod-induced mouse model, a strong skin infiltration of neutrophils and inflammatory monocytes can be observed. However, to date, it is not clear how exactly those cellular populations are attracted to the skin and how they contribute to the pathogenesis of the disease. To define the crucial cell type responding to IL-17 and initiating the downstream pathology in psoriasis-like dermatitis, we used mice specifically lacking the IL-17 receptor (IL-17RA) in different cell types. Deletion of IL-17RA in T cells or myeloid had no impact on disease development. Only deletion of this receptor in keratinocytes reflected the full-body deletion of IL-17RA, resulting in strongly reduced dermatitis development. Imiquimod treatment of those IL-17 signaling–deficient mice maintained high monocyctic infiltration but failed to attract neutrophils into the skin. We conclude that keratinocytes are a critical cellular target for IL-17A–mediated neutrophil attraction and psoriasis development.

INTRODUCTION
Psoriasis is a chronic inflammatory skin disease characterized by skin tissue infiltration of T cells, monocytes, and neutrophils that are associated with strong hyperproliferation of keratinocytes (KCs) and parakeratosis. Several cytokines have been implicated in the pathogenesis of the disease, including tumor necrosis factor-α (TNFα), IL-17A, IL-23, and IL-22 (Di Cesare et al., 2009), and neutralizing therapies against IL-17A, TNFα, or IL-12/IL-23-p40 are highly efficient in the treatment of patients (Kurschus and Moos, 2017).

The IL-17 family consists of six members (IL-17A–F), of which four cytokines, namely, IL-17A, -F, -C, and -E, all signal through a heterodimeric receptor containing the IL-17 receptor chain A (IL-17RA) (Toy et al., 2006; Yao et al., 1995). Of these, IL-17A and its most homologous family member, IL-17F, signal through to a receptor complex that includes not only IL-17RA but also IL-17RC. Although IL-17RA is expressed ubiquitously (Yao et al., 1995), expression of IL-17RC is rather restricted to epithelial cell types (Haudenschild et al., 2002; Kuestner et al., 2007). Nevertheless, several groups reported signaling of different IL-17 members on macrophages, T cells, or neutrophils (Ishigame et al., 2009; Taylor et al., 2014). Signaling by IL-17 in synergy with TNFα induces not only antibacterial defensins and cytokines but also chemokines such as CXCL1, CXCL2, and CXCL8, which mediate neutrophil tissue infiltration (Ye et al., 2001).

Psoriasis can be modeled in mice by daily topical application of Aldara cream containing 5% imiquimod (IMQ) (Meda, Solna, Sweden). The IMQ-induced dermatitis model phenocopies important features of psoriasis, including scaling, erythema, and acanthosis (van der Fits et al., 2009), as well as gene expression profiles that resemble those of psoriatic patients (Swindell et al., 2011). Similar to psoriasis, IL-17 signaling plays an important role in this model (El Malki et al., 2013; van der Fits et al., 2009). However, in contrast to psoriasis patients treated with IL-17 pathway–neutralizing drugs, who often show a complete remission (Langley et al., 2014; Papp et al., 2012; Waisman, 2012), mice deficient for IL-17RA are not fully resistant to disease development but show residual disease scores (El Malki et al., 2013). In line with this, it was recently shown that IL-17–independent pathways also play a role in this model (Walter et al., 2013). Nevertheless, a major infiltration of neutrophils and activated monocytes/macrophages and an elevation of T helper type 17 cells are also found in the IMQ model (El Malki et al., 2013; van der Fits et al., 2009). Additionally, many features of KC malfunctions, like hyperproliferation and disturbed epidermal differentiation, are similarly present in the IMQ model (van der Fits et al., 2009).

We used this model to address exactly how, and by which cell types and pathways, IL-17 initiates disease. We showed that cell type–specific deletion of the IL-17RA in KCs results in reduced IMQ-induced diseases, similar to what is seen in mice that lack this receptor altogether. In contrast, once IL-17RA was removed in T cells, neutrophils, or macrophages, no change was seen in disease severity, indicating that these cells are not (direct) relevant targets for IL-17.
amelioration in mice lacking IL-17RA in keratinocytes was paralleled by decreased IL-1, IL-22, and CXCL2 expression levels and diminished neutrophil infiltration into the skin. Nevertheless, these mice still showed high monocyte infiltrations into their skin, indicating that their role is most likely secondary in pathology perpetuation.

RESULTS

IL-17RA expression by T cells, neutrophils, and macrophages is dispensable for development of IMQ-induced dermatitis

We recently showed that full-body deletion of IL-17RA leads to reduced signs of IMQ-induced psoriasis in mice (El Malki et al., 2013). To investigate which individual cell type is the target of IL-17 in this model for psoriasis, we crossed the IL-17RAfl/fl line (El Malki et al., 2013) to different tissue-specific Cre lines. Mice expressing CD4-Cre crossed to the IL-17RAfl/fl strain lack IL-17RA in all αβ T cells due to deletion in the CD4⁺CD8⁺ double-positive stage during thymic maturation (Lee et al., 2001). We treated these mice, termed IL-17RACD4-Cre, with IMQ and analyzed the development of dermatitis over a time frame of 6 days, using Cre-negative littermates (IL-17RAfl/fl) as controls. We found no change in disease susceptibility of the IL-17RACD4-Cre mice compared with the control animals (Figure 1a). As expected, mice with full-body deficiency of IL-17RA (IL-17RAdel/del) were mostly protected from disease development, and mice treated with a cream not containing IMQ (sham) stayed normal in all parameters (Figure 1a). To elucidate whether IL-17 signaling in myeloid cells such as neutrophils or macrophages plays a role in IMQ-induced dermatitis, we crossed IL-17RAfl/fl mice to LysM-Cre-expressing mice, which were shown to induce recombination in 100% of neutrophils and in about 80%–90% of macrophages (Clausen et al., 1999). Treatment of the resulting IL-17RALysM-Cre mice with IMQ showed that dermatitis occurred independent of IL-17RA expression in neutrophils and macrophages (Figure 1b). We conclude that IL-17 signaling by T cells or neutrophils and macrophages does not play a critical role in the development of IMQ-induced dermatitis, in contrast to what we observed in mice with full-body deletion of IL-17RA.

KCs are the critical responder cells to IL-17 signaling after IMQ treatment

Because KCs were shown to respond to IL-17 signaling in psoriasis (Perera et al., 2012) and in the IMQ model (Ha et al., 2014), we next tested the impact of deleting IL-17RA in KCs by crossing the IL-17RAfl/fl mice to K14-Cre mice. The results showed that IMQ-induced dermatitis was not altered in IL-17RAfl/fl K14-Cre mice compared with IL-17RAfl/fl mice, whereas IL-17RAdel/del K14-Cre mice showed a marked reduction in disease development (Figure 1b). This indicates that KCs are the critical responder cells to IL-17 signaling after IMQ treatment.

Figure 1. Development of IMQ-induced dermatitis does not depend on IL-17RA in T cells, neutrophils, or macrophages. Mice were treated for 5 days with Aldara cream (5% IMQ) or sham cream, and their back skin was scored daily for 6 days. (a) Single (erythema, scaling, and thickness) and cumulative scores of IL-17RAfl/fl, IL-17RAdel/del, and IL-17RACD4-Cre mice. The scores are representative of two independent experiments. Sham-treated groups, n = 1; IMQ-treated groups, n = 3–5. (b) Single (erythema, scaling, and thickness) and cumulative scores of IL-17RAfl/fl and IL-17RAlysM-Cre mice. Sham-treated groups, n = 3; IMQ-treated groups, n = 5–6. *P ≤ 0.05 **P ≤ 0.01, ***P ≤ 0.001. IL-17RA, IL-17 receptor chain A; IMQ, imiquimod.
expressing mice (Hafner et al., 2004). After application of IMQ of IL-17RA\textsuperscript{K14-Cre} mice, we found that all investigated parameters, including erythema formation due to vasodilation, scaling, and thickening caused by acanthosis, were strongly reduced compared with the control group (Figure 2a and b). The remaining disease score of IL-17RA\textsuperscript{K14-Cre} mice was very similar to that observed in IL-17RA\textsuperscript{del/del} mice, suggesting that IL-17 signaling in keratinocytes is crucial for disease development in this model (Figure 2a and b). Histological analysis of the back skin showed that both cohorts, the mice lacking IL-17RA in KCs or in the whole body, showed massively reduced...
histopathological features of psoriasis (Figure 2c and d). Immune cell infiltrates of the epidermis, degradation of the epidermal structure, hyperproliferation of KCs, and para-keratosis leading to acanthosis were also significantly decreased compared with the IMQ-treated control mice (Figure 2c and d). Similarly, we found that IMQ-treated ears of IL-17RA<sup>K14-Cre</sup> and IL-17RA<sup>del/del</sup> mice showed significantly less thickening compared with IMQ-treated control mice (Figure 2e), concomitant with less acanthosis, immune cell infiltration, and hyperproliferation of KCs (Figure 2f and g). We conclude that IL-17RA signaling on KCs is crucial for development of IMQ-induced dermatitis because the conditional deletion of IL-17RA in KCs mimics the reduced disease severity of the body-wide IL-17RA–deficient mice.

**Neutrophil, but not monocyte, infiltration to IMQ-treated tissue depends on IL-17RA expression in KCs**

Triggering of the IL-17 signaling pathway was shown to result in the attraction of inflammatory myeloid cells to the site of the cytokine production (Ye et al., 2001). Therefore, we investigated cell infiltration in the ears of the IMQ-treated mice by flow cytometry. Analysis of ear infiltrates showed a strong infiltration of CD11b<sup>+</sup> cells in IMQ-treated control mice compared with sham-treated control mice, whereas both IMQ-treated IL-17RA<sup>del/del</sup> and IL-17RA<sup>K14-Cre</sup> mice displayed a reduced level of myeloid CD11b<sup>+</sup> cell infiltration compared with IMQ-treated controls (Figure 3a and b). Further analysis of the CD11b-positive myeloid cell population showed that neutrophil (Ly6C<sup>int</sup>/Ly6G<sup>+</sup> cells) numbers and relative percentages in the mononuclear populations were massively enhanced compared with the respective sham-treated controls (Figure 3c and d). We found significantly lower numbers of infiltrating neutrophils in the ears of the IMQ-treated IL-17RA<sup>del/del</sup> and IMQ-treated IL-17RA<sup>K14-Cre</sup> mice compared with IMQ-treated control mice. In contrast to neutrophils, infiltration of Ly6C<sup>+</sup>Ly6G<sup>−</sup> monocytes did not show any dependency of IL-17RA signaling, because the IL-17RA<sup>del/del</sup> and IL-17RA<sup>K14-Cre</sup> mice showed comparable infiltrates as the control mice (Figure 3c and d). Therefore, the found reductions in CD11b-positive myeloid cells are due to the lack of infiltrating neutrophils in IL-17RA<sup>del/del</sup> and IL-17RA<sup>K14-Cre</sup> mice but are not caused by the lack of monocyte infiltration. We further analyzed myeloid cell infiltration by immunofluorescence staining using myeloperoxidase as a marker for neutrophils and F4/80 as marker for inflammatory monocytes and macrophages to the IMQ-treated back skin. Similar to the flow cytometry data, we found that neutrophils were highly present in control mice but scarce in mice lacking IL-17RA in KCs or in the whole body, whereas no difference in F4/80<sup>+</sup> cells could be observed between these mouse strains versus the control animals (Figure 3e). These findings show that attraction of neutrophils, but not of monocytes, to the IMQ-treated tissue is dependent on IL-17 signaling in keratinocytes, suggesting that secondary cytokines and chemokines, which are responsible for neutrophil attraction to the IMQ-treated tissue, are either directly or indirectly produced by IL-17–activated keratinocytes in this model.

**Absence of IL-17RA signaling in KCs results in reduced expression of neutrophil-attracting stimuli**

To test whether neutrophil attraction to IMQ-treated tissue depends on IL-17 signaling in keratinocytes, we performed expression analysis for a selected set of cytokines, chemokines, and chemokine receptors previously implicated in neutrophil infiltration and psoriatic inflammation. We found that the expression of the chemokine Cxcl2 and of the chemokine receptor Ccr1 was up-regulated only in control mice treated with IMQ but not in the IL-17RA signaling–deficient mice (Figure 4). Both CXCL2 and CCR1 were previously shown to be important in the attraction of neutrophils to sites of inflammation. Similarly, expression of Il1a and Il1b was up-regulated after IMQ treatment in controls but only very little in the skin of IL-17RA<sup>K14-Cre</sup> and IL-17RA<sup>del/del</sup> mice. IL-22, a cytokine that was shown to be expressed specifically by T cells and innate lymphoid cells of group 3, was also highly expressed in the IMQ-treated control group but not the IL-17RA mutant mice.

Our results show that IL-17 signaling by KCs is responsible for the influx of neutrophils to the skin and to the pathogenesis of psoriasis-like disease in mice, thus making KCs a preferable cell type for manipulation in a clinical setting in psoriasis patients.

**DISCUSSION**

We investigated the target cell type for IL-17 in a mouse model for psoriasis and found that KC expression of IL-17RA is critical for the full induction of skin inflammation. Mechanistically, we showed that the attraction of neutrophils to the skin was prevented in the absence of IL-17 signaling in KCs. In contrast, we found that monocytic infiltration into the skin was independent of IL-17 signaling in KCs.

What exactly triggers the inflammation in the skin of psoriasis patients is not clear. One possibility is that T cells recognize an autoantigen in the skin, and after their activation, they secrete key cytokines such as IL-17A, IL-22, and TNFα (Prinz, 2018). These cytokines trigger the local cells, such as the KCs, leading to the production of chemokines that initiate neutrophil infiltrations to the skin. As described, IMQ-induced dermatitis in mice only partially models the disease in humans, one major difference being its distinct etiology: by skin irritation via TLR7 activation (after IMQ treatment) versus a possible autoimmune etiology for plaque psoriasis. Nevertheless, the local presence of high IL-17A levels and similar downstream events in the tissue such as neutrophil attraction are common features of both psoriasis and the IMQ model.

To understand the pathology of psoriasis and the effectiveness of its treatment by IL-17 pathway–neutralizing drugs, it is essential to understand how IL-17A exerts its harmful action. In psoriasis, not only KCs but also fibroblast or endothelial cells may be targets of IL-17. Even though it is thought that IL-17RC is not expressed on hematopoietic cells, a direct reaction of macrophages, neutrophils, and T cells to IL-17A has been described (Ishigame et al., 2009; Taylor et al., 2014). We were able to exclude T cells and macrophages/neutrophils as IL-17 targets in psoriasis-like disease, and although we did not formally prove that KCs are the only target cell population important for the development of...
Figure 3. Infiltration of neutrophils, but not of monocytes, to IMQ-treated tissue depends on IL-17RA on keratinocytes. (a–d) Flow cytometric analysis of myeloid cell infiltrates into sham- or IMQ-treated ears of IL-17RA^{fl/fl}, IL-17RA^{del/del}, and IL-17RA^{K14-Cre} mice on day 6. Sham-treated groups, n = 4; IMQ-treated groups, n = 3–5. (a) Dot plot showing CD11b^{+} gated cells. (b) Quantification of CD11b^{+} cells, shown as the percentage of living cells and as total cell numbers.
IMQ-mediated dermatitis, our data strongly suggest that KCs are the major important target cells for the IL-17 response in this model.

A recent study showed that depletion of neutrophils in the IMQ model lowered disease development similarly as seen here by the use of IL-17R<sup>del/del</sup> or IL-17RA<sup>K14-Cre</sup> mice (Sumida et al., 2014). Neutrophils, which are often found in plaque psoriasis in the upper epidermis layers in the form of Munro microabscesses (Perera et al., 2012), may propagate disease by increasing neovascularization. It was shown that neutrophils secrete VEGF in response to G-CSF exposure (Ohki et al., 2005). This is in line with a strong decrease in neutrophils secrete CXCL1 and CXCL2, which attract neutrophils in the skin in the absence of neutrophils (Boniface et al., 2005; Ma et al., 2008; Wolk et al., 2006). In line with that, we recently showed that the IL-22 inhibitor IL-22BP is down-regulated in skin of psoriasis patients compared with skin from healthy patients, enabling IL-22’s function in the disease (Voglis et al., 2018). Here, we found that the cytokine IL-22 was not up-regulated in mice lacking an IL-17 response in KCs after IMQ treatment. It is possible, therefore, that IL-17–activated KCs induce the infiltration of IL-22—producing cells or their generation in situ.

In summary, our data show that KCs are the main target cells for IL-17 action in the skin in psoriasisform dermatitis, steering neutrophil infiltration and downstream pathology.

**MATERIALS AND METHODS**

**Mice**

Experiments were performed with the indicated mouse lines. To prevent signaling of IL-17RA in specified tissues, IL-17RA<sup>fl/fl</sup> mice with the exons 4–7 flanked by loxP sites (El Malki et al., 2013) were crossed to the following Cre lines: CD4-Cre (Lee et al., 2001), LysM-Cre (Clausen et al., 1999), or K14-Cre (Hafner et al., 2004). To obtain body-wide deletion of IL-17RA, the floxed mice were crossed to a Deleter-Cre–expressing line (Schwenk et al., 1995). All mice were generated on C57BL/6 genetic background or backcrossed at least 10 times. The mice were bred and housed in the animal facility of the University Medical Center of the Johannes Gutenberg-University of Mainz under specified pathogen-free conditions. All mouse experiments were carried out in accordance with the relevant guidelines and regulations for animal welfare by the federal state of Rhineland-Palatinate, Germany. Experiments were done with approval from the Landesuntersuchungsamt Rheinland-Pfalz (individual animal experimentation application no. G13-1-096), and all efforts were made to minimize suffering of the mice.

**IMQ-induced psoriasis mouse model**

Mice were depilated on the back skin 2 days before the treatment and then daily treated with either Aldara (containing 5% IMQ, purchased from Meda [Solna, Sweden]) or sham cream on the back skin and ears for 5 or 6 consecutive days. For the back skin, disease severity was assessed every day with a scoring system considering scaling, erythema, and skin thickness, similar to the human Psoriasis Area and Severity Index, but not taking the area into account because it is defined by the experimenter. A cumulative score was
generated from the three mentioned parameters. Erythema and scaling were scored from 0 to 4, with 0 indicating no severity and 4 indicating high severity. Thickness of the skin was scored based on the increase in the thickness compared with day 1 (1 for 20%–40%, 2 for 40%–60%, 3 for 60%–80%, and 4 for >80%). Additionally, ear thickness was measured every day.

**Flow cytometry**

Ears were mechanically disrupted and digested with 0.25 mg/ml Liberase (Roche, Basel, Switzerland) and 0.05 mg/ml DNase (Roche). Subsequently, cells were filtered through a 40-μm cell strainer (BD Falcon, San Jose, CA). The resulting single cell suspension was incubated with Fc-blocking reagent (BioXCell, West Lebanon, NH) before the surface staining with the following fluorescence-conjugated antibodies: αCD11b (clone: M1/70; eBi-science, San Diego, CA), αLy6C (clone: AL-21; BD Biosciences, San Jose, CA) and αLy6G (clone: 1A8; BioLegend, San Diego, CA). Dead cells were excluded using Fixable Viability Dye eFluor 780 (Thermo Fisher Scientific, Waltham, MA). Stained cells were acquired using a BD FACS Canto II and analysis was performed using FlowJo (Ashland, OR) software.

**Histology**

For hematoxylin and eosin staining, tissue samples were fixed in 4% formaldehyde and embedded in paraaffin, and sections of 5–6 μm were cut before the staining was performed. Images were taken using the microscope IX81 (Olympus, Shinjuku, Japan). For immunohistochemistry, cryo sections of 7–8 μm were cut. The following antibodies were used: primary antibodies: α-myoeloperoxidase (polyclonal; Abcam, Cambridge, UK) and αF4/80 (clone: BM8, eBi-science, San Diego, CA), secondary antibodies: goat α-Rat (polyclonal; Sigma-Aldrich, St. Louis, MO), and goat α-Rabbit (polyclonal, Sigma-Aldrich). Nuclei were counterstained with Hoechst 33342 (Invitrogen, Waltham, MA). Images were taken using the fluorescence microscope DMi8 (Leica, Wetzlar, Germany).

**RNA isolation of skin tissue and quantitative real-time PCR**

RNA from ear tissue was extracted using TriFast (VWR International, Radnor, PA), and cDNA was synthesized with the Quantitect Reverse Transcription Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Genes of interest were quantified with GoTaq qPCR Master Mix (Promega, Madison, WI), as the reference gene. All primers were purchased from scilife (clone: BM8, eBiosciences, San Diego, CA), secondary antibodies: goat α-Rat (polyclonal; Sigma-Aldrich, St. Louis, MO), and goat α-Rabbit (polyclonal, Sigma-Aldrich). Nuclei were counterstained with Hoechst 33342 (Invitrogen, Waltham, MA). Images were taken using the fluorescence microscope DMi8 (Leica, Wetzlar, Germany).

**Statistical analysis**

Graphs were created using GraphPad Prism (GraphPad Software, San Diego, CA). Statistical significance was calculated with one-way analysis of variance with Bonferroni post hoc test. Values of $P \leq 0.001$, $P \leq 0.01$, and $P \leq 0.05$ were considered statistically significant. Data are represented as means with standard deviations.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

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