IL-6 Regulates Neutrophil Microabscess Formation in IL-17A-Driven Psoriasiform Lesions

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The lack of a generally accepted animal model for human psoriasis has hindered progress with respect to understanding the pathogenesis of the disease. Here we present a model in which transgenic IL-17A expression is targeted to the skin in mice, achievable after crossing our IL-17A^{ind} allele to the K14-Cre strain. K14-IL-17A$^{ind/+}$ mice invariably develop an overt skin inflammation bearing many hallmark characteristics of human psoriasis including dermal infiltration of effector T cells, formation of neutrophil microabscesses, and hyperkeratosis. IL-17A expression in the skin results in upregulated granulopoiesis and migration of IL-6R-expressing neutrophils into the skin. Neutralization of IL-6 signaling efficiently reduces the observed pathogenesis in skin of IL-17A-overexpressing mice, with marked reductions in epidermal neutrophil abscess formation and epidermal thickening. Thus, IL-6 functions downstream of IL-17A to exacerbate neutrophil microabscess development in psoriasiform lesions.

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INTRODUCTION

Psoriasis is a disease characterized by increased epidermal thickness (acanthosis), rapid proliferation of keratinocytes, altered keratinocyte differentiation, an abnormal collection of polymorphonuclear leukocytes in the epidermis, and an activated mononuclear cell infiltrate in the underlying dermis (Nograles *et al.*, 2009). It is a chronic, inflammatory, immune-mediated skin disease, affecting approximately 1–3% of the population worldwide associated with extensive psychological and physical burdens (Greaves and Weinstein, 1995). To explain the molecular pathology of this disease, one needs to consider potential roles for cytokines that both influence epidermal growth and regulate cellular immune activation and inflammation. A significant amount of both clinical and experimental data have established T helper type 17 (Th17) cells as key players in chronic inflammatory conditions such as psoriasis (Waisman, 2012), providing a significant advancement of our understanding of the immunopathogenesis of psoriasis and potential targets for therapeutic agents (Di Cesare *et al.*, 2009). This is also supported by the observation that mice deficient in crucial IL-17 signaling components show reduced psoriasiform plaque formation compared with control mice following topical application of Aldara, an imiquimod-containing cream also used to mimic features of psoriasis in mice (van der Fits *et al.*, 2009; Pantelyushin *et al.*, 2012; El Malki *et al.*, 2013).

Pro-inflammatory Th17 cells have been shown to be present in lesions from both psoriatic patients and chemically induced models of psoriasiform dermatitis in mice (Harper *et al.*, 2009; van der Fits *et al.*, 2009). IL-17A-producing cells have also been isolated from the dermis of psoriatic lesions (Lowes *et al.*, 2008). Teunissen *et al.* (1998) first described IL-17A as a potential mediator in psoriasis after identifying upregulated IL-17A mRNA expression in lesional psoriatic skin. CD4 and CD8 clones derived from psoriasis lesions were also able to produce IL-17A after TCR stimulation.

The Th17-supporting cytokine IL-23 was demonstrated to promote the production of IL-17F and tumor necrosis factor-α from primed T lymphocytes (Aggarwal *et al.*, 2003), and psoriasis-like phenotypes can be artificially induced in mouse

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Abbreviations: MCP-3, monocyte chemotactic protein-3; Th17, T helper type 17

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skin using intradermal injections of IL-23 (Hedrick *et al.*, 2009). The results of the clinical studies on the efficacy of anti-IL-12p40 antibodies observed so far have yielded good results. Both ustekinumab and ABT-874, two human IgG1 monoclonal antibodies directed against the p40 subunit of IL-23 and IL-12, provided effective treatment for patients with moderate-to-severe psoriasis (Di Cesare *et al.*, 2009; Nestle and Conrad, 2004). Thus, the important role of the IL-23/IL-17A axis in the pathogenesis of the disease is becoming well established. In addition to IL-17A signaling molecules being genetically associated with psoriasiform pathology (Ellinghaus *et al.*, 2010; Hoeve *et al.*, 2006), associations of other pro-inflammatory regulators such as IL-6 have also been shown (Toruniowa *et al.*, 1995; Arican *et al.*, 2005).

In order to further analyze the correlation between IL-17A and the development of psoriasis, we generated a mouse strain in which IL-17A and enhanced green fluorescent protein are co-expressed in keratinocytes using a Cre-LoxP approach resulting in a psoriasis-like lesion formation. The ensuing pathogenesis bore many hallmark features of human psoriasis including hyperkeratosis, parakeratosis, epidermal neutrophil accumulation and formation of microabscesses, scaling, erythema and thickening of the skin. Here we characterize the skin disease, systemic effects, and comorbidities of this mouse strain. We could observe that both Ly6G^{hi} neutrophils and Ly6C^{hi} monocytes strongly express the IL-6Rα in bone marrow and blood and before their subsequent migration into the inflamed skin. Blockade of IL-6 signaling using an mAb efficiently reduced the skin pathology of K14-IL-17A$^{ind/+}$ mice, with strongly reduced formation of neutrophil micro-abscesses in the epidermis and a marked reduction in myeloperoxidase^{+} cells in skin sections. Therefore, despite a role for IL-6 in neutrophil clearance to resolve inflammation (McLoughlin *et al.*, 2003), IL-6 signaling is an essential pathway by which neutrophils mediate pathogenicity in the inflamed skin tissue.

RESULTS

Skin-specific IL-17A expression induces severe psoriasis-like skin inflammation in mice

To allow for in-depth analysis of psoriatic inflammation in a mouse model, we generated a previously unreported strain that closely mimics hallmark features of human psoriasis acknowledging the role of IL-17 in the disease pathogenesis. The IL-17A^{ind} allele allows for conditional overexpression of IL-17A and enhanced green fluorescent protein following Cre-mediated recombination (Figure 1a; Haak *et al.*, 2009). Crossed to the keratinocyte-specific K14-Cre (Hafner *et al.*, 2004; hereafter termed K14-IL-17A$^{ind/+}$), these mice display dry and flaking skin shortly after birth (data not shown). After reaching maturity, K14-IL-17A$^{ind/+}$ mice develop a remarkably consistent skin inflammation comparable to severe human psoriasis (Figure 1b). Histological examination of skin samples obtained from the back skin of K14-IL-17A$^{ind/+}$ mice and littermate controls revealed an acanthotically thickened epidermis, loss of the stratum granulosum, and an elongation of the papillary dermis as well as areas of hyper- and parakeratosis and multiple neutrophilic abscesses in the horny layer (Figure 1c). We were also able to observe clinical signs of uveitis and arthritis, which represent typical comorbidities of psoriasis (Lambert and Wright, 1976; Farley and Menter, 2011; Supplementary Figure 1a and b online). No signs of colitis were observed when compared with RAG1-deficient mice receiving naive T cells (Supplementary Figure 1c online).

Skin disease progression was monitored using the psoriasis area and severity index adapted to mice over 10 weeks after onset showing a strong impairment of the skin compared with control mice (Figure 1d). Although no skin inflammation was detectable in IL-17A$^{ind/+}$ littermates, all K14-IL-17A$^{ind/+}$ mice develop a spontaneous inflammation reminiscent of human psoriasis. Given the well-known link between signaling of IL-17A in infected tissue and granulopoiesis (Kolls and Linden, 2004), we reasoned that K14-IL-17A$^{ind/+}$ mice would also show a phenotype here. We detected a significant increase in the population of granulocytes in the bone marrow, as seen by staining with the surface markers CD11b and Gr-1 (Figure 1e). In the blood of K14-IL-17A$^{ind/+}$ mice, an overt mobilization of Ly6G^{hi}CXCR2^{hi} cells was observed, as was an increase in CD115^{+}Ly6C^{hi} monocytes (Figure 1f). Further analysis confirmed that this increase was significant for both neutrophils and monocytes (Figure 1g), suggesting that IL-17A expression in the skin led to mobilization of these cells from the bone marrow to the skin via the blood stream. We found no difference in the percentage of eosinophils in the blood, but we did detect a small reduction in the percentage of lymphocytes in the blood of K14-IL-17A$^{ind/+}$ mice, reflecting the relative increase in that of monocytes and neutrophils (Figure 1g).

Granulocytes, macrophages, and effector T cells invade the skin of K14-IL-17A$^{ind/+}$ mice

Skin from K14-IL-17A$^{ind/+}$ mice showed clustering of myeloperoxidase-expressing cells in the inflamed epidermis, indicative of neutrophil microabscess formation (Figure 2a), as well as F4/80^{+} cells accumulating in the dermis of inflamed skin (Figure 2b). Significantly elevated levels of IL-17A, macrophage inflammatory protein-1β, monocyte chemotactic protein-3 (MCP-3), GM-CSF, RANTES, and IL-6 were detectable in supernatants collected from whole skin cultures of K14-IL-17A$^{ind/+}$, but not of control mice (Figure 2c). Functional IL-17A signaling was further substantiated by highly elevated levels of CCL2/monocyte chemotactic protein-1 protein in skin, accounting for the influx of innate cells types (Figure 2d).

Significantly upregulated expression of genes encoding the lineage-defining transcription factors T-bet, RORγT and Foxp3 were detected in K14-IL-17A$^{ind/+}$ skin, highlighting the presence of both effector T cells of Th1 and Th17 phenotype in addition to regulatory T cells (Figure 2e). We wanted to further determine if these transcription factors belonged to the $\alpha\beta$ or $\gamma\delta$ T cells lineage. Interestingly, inflamed epidermis and dermis from K14-IL-17A$^{ind/+}$ skin was almost completely devoid of $\gamma\delta$ T cells compared with IL-17 A$^{ind/+}$ control skin, where the CD11b^{-} $\gamma\delta$ TCR^{+} cell type represents skin-resident dendritic epidermal gamma/delta T cells (Figure 2f). Instead, an elevated proportion of TCRβ^{+} cells can be seen in the epidermis of K14-IL-17A$^{ind/+}$ skin. Both dermis and epidermis

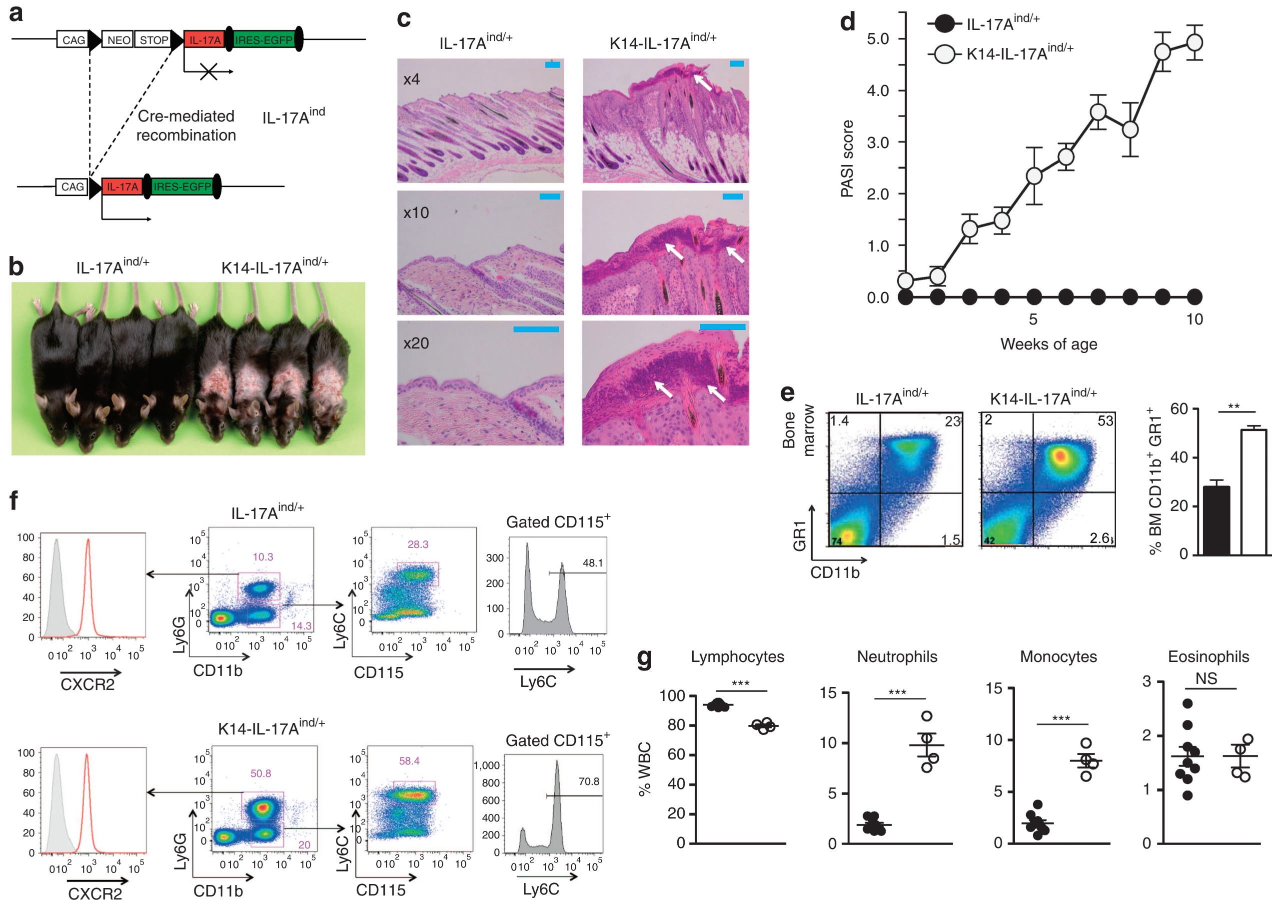

Figure 1. Overexpression of IL-17A in the skin results in a psoriasis-like phenotype. (**a**) Cre-mediated conditional overexpression of IL-17A and enhanced green fluorescent protein (EGFP) using the IL-17A^{ind} allele. (**b**) Six-week-old IL-17A$^{ind/+}$ and K14-IL-17A$^{ind/+}$ mice are depicted. The consistency and placement of lesion development are shown. (**c**) Hematoxylin and eosin (H&E) stainings of lesional back skin sections from IL-17 A$^{ind/+}$ and K14-IL-17A$^{ind/+}$ mice ($n=3$). White arrows: neutrophil microabscess formation. Blue scale bars: 4 × = 50 μm, 10 × = 50 μm, 20 × = 100 μm. (**d**) Psoriasis area and severity index (PASI) modified from the human PASI guidelines to score erythema, scaling, skin thickness, and affected area in mice ($n=5$). (**e**) Bone marrow of K14-IL-17A$^{ind/+}$ mice was stained for CD11b and GR1. Populations of GR1$^+$CD11b$^+$ cells in each compartment are shown with representative statistics (mean ± SEM) and the corresponding significance ($n=3$). (**f**) Blood from IL-17A$^{ind/+}$ and K14-IL-17A$^{ind/+}$ mice ($n=2$) was red blood cell-lysed and stained for CD11b, CXCR2, Ly6G, Ly6C, and CD115. Gating pathway is indicated with arrows and percentages in the quadrants or histograms are given. (**g**) Percentages of total white blood cells (WBCs) were determined after performing a routine complete blood count analysis. Fractions of lymphocytes, monocytes, neutrophils, and eosinophils are shown with indicated significance. Each panel is representative of at least two experiments with consistent results. *$P<0.05$; **$P<0.01$; ***$P<0.001$. Significance was calculated using Student's *t*-test.

showed a clear increase in CD11b^{hi}Ly6G$^+$ cells, indicative of a robust neutrophil migration into the IL-17A-expressing skin tissue. The thickening of the epidermis of the ear is also clearly visible by histology (Figure 2g).

Anti-IL-6 treatment reduces IL-17A-induced neutrophil microabscess formation

Anti-IL-6 treatment has been shown to be effective in reducing clinical symptoms in diseases such as rheumatoid arthritis (Maini *et al.*, 2006). In addition, neutrophil counts were reduced in a dose-dependent manner after tocilizumab administration, an mAb designed to neutralize human IL-6 (Maini *et al.*, 2006). We therefore reasoned that IL-6 may be important downstream of IL-17A to increase the severity of inflammation in K14-IL-17A$^{ind/+}$ mice. We detected increased inducible nitric oxide synthase in the skin of the K14-IL-17A$^{ind/+}$ mice, which indicates an active innate immune compartment (Figure 3a). However, no difference was observed in total expression of IL-23p19 (Figure 3a). This further supports the idea that IL-23 acts upstream of IL-17A production and indicates that the severity of the inflammation observed is not influenced by a secondary infection in lesional skin. This increased level of IL-6 expression was not confined to the inflamed skin, as highly elevated levels of IL-6 accompanied the elevated levels of IL17A also in the serum of K14-IL-17A$^{ind/+}$ mice (Figure 3b). Interestingly, in addition to elevated serum levels of IL-6, neutrophils, and monocytes in the blood express IL-6Rα in both IL-17A$^{ind/+}$ and K14-IL-17A$^{ind/+}$ mice, detectable by presence of CD126 on both Ly6G^{hi} and Ly6C^{hi} cells in both blood and bone marrow (Figure 3c).

To test the importance of IL-6 signaling in IL-17A-induced psoriasis, we treated K14-IL-17A$^{ind/+}$ mice with either

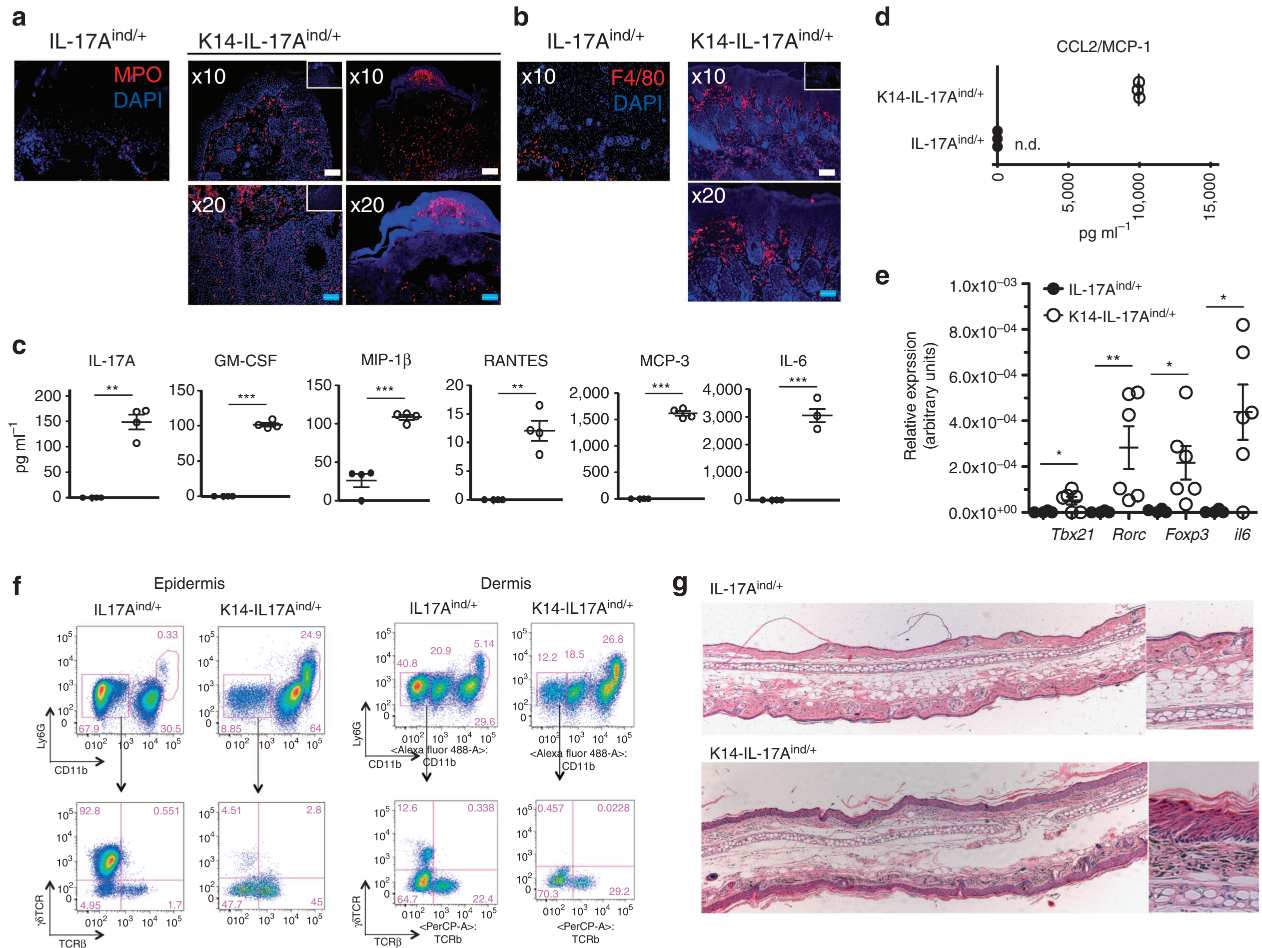

Figure 2. Skin-specific and systemic effects of the local IL-17A overexpression in the skin. (**a**, **b**) Skin of K14-IL-17A$^{ind/+}$ and control mice was stained by fluorescence-immunohistochemistry for myeloperoxidase (MPO)$^{+}$ and F4/80^{+} (magnifications are given from representative stainings, white scale bars = 100 μm, blue scale bars = 50 μm; $n=3$ or more). (**c**) Cell suspensions from IL-17A$^{ind/+}$ or K14-IL-17 A$^{ind/+}$ skin were cultured. Chemokine concentration in the supernatants after 24 hours was measured by flow cytomix for CCL2/monocyte chemotactic protein-1 (MCP-1), IL-17A, MCP-3, macrophage inflammatory protein (MIP)-1β, RANTES, GM-CSF, and IL-6 ($n=4$). (**d**) As in (**c**), with measurements for total CCL2/MCP-1 in supernatant shown ($n=3$). (**e**) Total RNA isolated from lesional back skin of either K14-IL-17A$^{ind/+}$ or control mice. Quantitative real-time reverse transcriptase–PCR (RT-PCR) for the indicated genes was performed. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as housekeeping gene for relative determinations. (**f**) Single-cell suspensions obtained from mechanically disrupted epidermis and dermis of K14-IL-17A$^{ind/+}$ and control mice were indicated markers after exclusion of dead cell exclusion and pre-gating on CD45^{+} cells. (**g**) Hematoxylin and eosin (H&E) histology was performed on ears of K14-IL-17A$^{ind/+}$ and control mice. Each panel is representative of at least two experiments with similar results. *$P<0.05$; **$P<0.01$; ***$P<0.001$. Significance was calculated using Student's *t*-test.

neutralizing antibody against IL-6, or a sham treatment (Hoge *et al.*, 2013). Despite a strong inflammation remaining in untreated K14-IL-17A$^{ind/+}$ skin, anti-IL-6 treatment efficiently reduced the severity of leukocyte infiltration with a dramatic reduction in neutrophil microabscess formation and scaling in the epidermis of K14-IL-17A$^{ind/+}$ skin (Figure 3d). Anti-IL-6 treatment also significantly reduced the epidermal thickness compared with K14-IL-17A$^{ind/+}$ mice receiving only a sham treatment (Figure 3e). We were also able to observe a reduced accumulation of myeloperoxidase^{+} cells in skin sections of K14-IL-17A$^{ind/+}$ mice after a prolonged treatment with anti-IL-6 (Figure 3f). This could be also reconfirmed by showing a significant reduction of the CD11b^{+} cells in lesional skin after anti-IL-6 treatment (Figure 3g). Interestingly, innate inflammatory infiltrates in the inflamed skin maintain expression of the IL-6Rα (Figure 3h). Thus, neutralization of IL-6 significantly reduced the severity of inflammation caused by transgenic overexpression of IL-17A in the skin. Given the reduced severity of inflammation and its correlation with reduced numbers of myeloperoxidase^{+} cells in the K14-IL-17A$^{ind/+}$ skin, we conclude that neutralization of IL-6 reduces innate-driven inflammation induced by aberrant IL-17A expression in the skin.

DISCUSSION

In this report, we have introduced a previously unreported mouse model well suited to effectively study the immunopathogenesis of psoriasis. The finding that IL-17A neutralization in the clinic has been efficacious certainly implies that an IL-17A-mediated animal model of psoriasis should accurately recapitulate the characteristics of human psoriasis (Girolomoni *et al.*, 2012; Patel *et al.*, 2013). Effector T-cell infiltration,

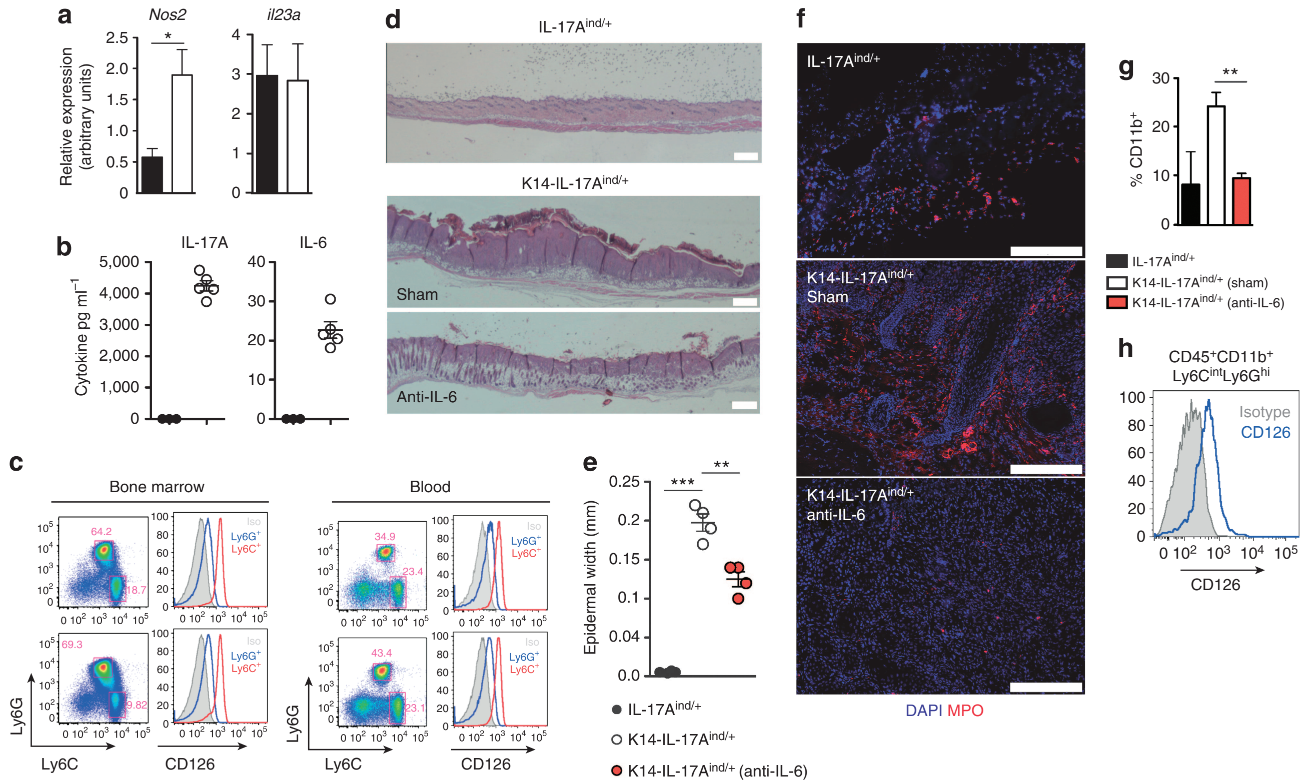

Figure 3. Neutralization of IL-6 reduces epidermal thickness and neutrophil accumulation in K14-IL-17A$^{ind/+}$ skin. (**a**) Total RNA isolated from back skin of either K14-IL-17A$^{ind/+}$ or control mice was analyzed by quantitative real-time reverse transcriptase–PCR (RT-PCR) for the indicated genes. Shown are relative expression levels normalized to hypoxanthine-guanine phosphoribosyltransferase. Closed bars represent IL-17A$^{ind/+}$; open bars represent K14-IL-17A$^{ind/+}$. (n = 5–7 mice). (**b**) IL-17A and IL-6 were measured in the serum of the indicated mice. Cytokines were not detectable in IL-17A$^{ind/+}$ control mice (IL-17A$^{ind/+}$, closed circles; K14-IL-17A$^{ind/+}$, open circles). (**c**) Blood and bone marrow was taken from K14-IL-17A$^{ind/+}$ or control mice. After red blood cell lysis, cells were analyzed by FACS for expression of the IL-6Rα chain, CD126. Shown are dot plots pre-gated on CD11b^{hi} cells with additional markers Ly6G and Ly6C indicated. (**d**) Mice were treated with either 25 μg/g body weight of anti-IL-6 twice a week for a 4-week period, or an equal volume of phosphate-buffered saline (PBS) used as a sham control. Treatment began at 2 weeks of age, and finished at 6 weeks of age. Hematoxylin and eosin (H&E) stainings were performed on skin sections from K14-IL-17A$^{ind/+}$ and IL-17A$^{ind/+}$ control mice (scale bars represent 200 μm). (**e**) Epidermal thickness was measured in IL-17A$^{ind/+}$, sham-treated K14-IL-17A$^{ind/+}$, or anti-IL-6 treated K14-IL-17A$^{ind/+}$ mice. Each data point represents the mean average of multiple measurements of epidermal width from individual mice either before or after treatment with anti-IL-6 (n = 4). (**f**) Skin of K14-IL-17A$^{ind/+}$ treated with anti-IL-6 or sham over 7–10 weeks and control mice was stained by fluorescence-immunohistochemistry for myeloperoxidase (MPO) and 4,6-diamidino-2-phenylindole (DAPI; scale bars represent 200 μm). Reduction of MPO$^+$ cells is visible after treatment with anti-IL-6 (n = 5). (**g**) Frequencies of CD11b$^+$ cells isolated from lesional back skin are shown after treatment of K14-IL-17A$^{ind/+}$ mice with anti-IL-6 over 7–10 weeks (n = 4). (**h**) Skin infiltrating granulocytes in K14-IL-17A$^{ind/+}$ skin were stained for CD126 or an isotype control (n = 2). In each panel, experiments have been repeated at least twice with similar results obtained. Statistical significance was calculated using Student's t-test and error bars represent the SEM. *P < 0.05; **P < 0.01; ***P < 0.001. Significance was calculated using Student's t-test.

neutrophil microabscesses, hyperkeratosis, and scaling are all present in K14-IL-17A$^{ind/+}$ skin. These features collectively distinguish the K14-IL-17A$^{ind/+}$ model from other attempts to transgenically express cytokines under the control of skin-specific promoters (Gudjonsson *et al.*, 2007).

High levels of IL-17A in organs expressing the IL-17R essentially mimics a bacterial or fungal infection, which in itself would result in generation of Th17 cells and the downstream inflammatory response, which includes a neutrophil influx (Kelly *et al.*, 2005; Aujla *et al.*, 2007; Zhou *et al.*, 2009; Cho *et al.*, 2010). As the IL-23/IL-17A axis was identified as a key facet in the pathogenesis of psoriasis (Shear *et al.*, 2008; Di Cesare *et al.*, 2009), much focus has been placed on IL-23, which is secreted by skin-resident dendritic cells and induces production of pro-inflammatory mediators by Th17 cells such as IL-17A, IL-17F, and IL-22. These cytokines signal to keratinocytes, causing activation, chemokine and antimicrobial peptide expression, and hyperproliferation. This chain of events ultimately results in an immune amplification, leading to the clinical features of the psoriasis. In the case of the K14-IL-17A$^{ind/+}$ strain, the inflammatory cascade begins with transgenic IL-17A expression in the skin, negating the requirement of IL-23. It also implies that IL-23 is required mainly for induction of Th17 cells and IL-17A expression, as the inflammation presented in this study continues even in the absence of upregulated IL-23 expression.

An important strength of this model is the T-cell infiltrates observed in skin of K14-IL-17A$^{ind/+}$ mice. The absence of T-cell infiltrates has been a concern in a number of previous mouse models for psoriasis-like skin disease (Gudjonsson *et al.*, 2007). This is an important difference to the TLR-7-dependent (or independent) Aldara/Imiquimod model (Walter

et al., 2013), where γδ T cells and IL-17 family cytokines are critical, but independent of effector αβ T-cell infiltrates seen in human psoriasis (Becher and Pantelyushin, 2012; Pantelyushin *et al.*, 2012). The observation of elevated T-bet and RORγT levels in the skin and an almost complete lack of infiltrating γδ T cells combined with increased $TCR\beta^+$ cells in the epidermis shows that some degree of effector T-cell migration to the skin is taking place. IL-17A has been shown to upregulate expression of CCL20 in keratinocytes (Harper *et al.*, 2009). Th17 cells express CCR6 (Hirota *et al.*, 2007; Harrison *et al.*, 2008; Wang *et al.*, 2009; Turner *et al.*, 2010), the receptor for CCL20 and by this mechanism could migrate into the inflamed skin of K14-IL-17A$^{ind/+}$ mice. Furthermore, macrophage inflammatory protein-1α and macrophage inflammatory protein-1β, both upregulated in K14-IL-17A$^{ind/+}$ skin (Figure 2a), are known to be potent chemo-attractants for effector T cells (Schall *et al.*, 1993; Taub *et al.*, 1993; Lillard *et al.*, 2003).

Given the important role of IL-6 in a number of inflammatory disease models including experimental autoimmune encephalomyelitis, collagen-induced arthritis and transfer colitis, we are not surprised to see a beneficial effect after neutralizing such a major player in inflammation (Neurath and Finotto, 2011; Rincon, 2012). IL-6 is secreted by a variety of cell types other than effector T cells, including fibroblasts, endothelial cells, hepatocytes, keratinocytes, and astrocytes, primarily in response to tissue injury or infection. IL-17A was shown to promote the production of IL-6, IL-8, GM-CSF, and ICAM-1 in keratinocytes (Albanesi *et al.*, 2000; Koga *et al.*, 2008). IL-6 has long been associated with lesional psoriatic skin (Castells-Rodellas *et al.*, 1992) and its role was suggested to be the enhancement of keratinocyte proliferation (Grossman *et al.*, 1989) and stimulation of the growth of keratinocytes (Kishimoto *et al.*, 1992). These results were recently supported by another publication claiming that IL-6 expression from human keratinocytes was induced by IL-17F, a Th17 cytokine (Fujishima *et al.*, 2010). IL-6 signaling on keratinocytes was previously shown to induce chemo-attractant proteins via AP-1, a downstream target gene following IL-6 signaling (Sano *et al.*, 2005; Zenz *et al.*, 2005). The IL-6 pathway has been shown to have a direct role in psoriatic lesion formation in another mouse model of psoriasis-like skin disease relying on constitutive activation of Stat3 in keratinocytes (Sano *et al.*, 2005; Zenz *et al.*, 2005). Besides the significant correlation between the serum IL-17 level and the disease severity in human psoriasis shown in a clinical study fitting to the elevated IL-17A levels, we detected in the K14-IL-17A$^{ind/+}$ mice (Arican *et al.*, 2005), psoriasis patients show increased levels of IL-6 in their serum as is also the case in the K14-IL-17A$^{ind/+}$ strain (Toruniowa *et al.*, 1995). IL-6 can also command monocyte differentiation into macrophages, increase oxidative burst and MCP-1, tumor necrosis factor-α, and IL-12 production (Kaplanski *et al.*, 2003). Bartoccioni *et al.* (2003) proposed that many cell types could use IL-6 secretion and trans-signaling to target monocyte chemotaxis and maintain sustained chronic inflammation. The immunopathogenesis that we see in the K14-IL-17A$^{ind/+}$ strain strongly supports this proposed mechanism, given a sustained presence of IL-6Rα-expressing monocytes and neutrophils in the inflamed skin. Indeed, IL-6 has been proposed to propagate IL-17A-induced inflammation in experimental autoimmune encephalomyelitis (Ogura *et al.*, 2008).

The chronic nature of this inflammation, given the relentless expression of IL-17A under the control of the CAG promoter, stimulates the target cell to produce and secrete IL-6, which can then bind to soluble IL-6 receptor α. The IL-6–soluble IL-6 receptor α heterodimer then can bind to glycoprotein 130 on the target cell membrane (Romano *et al.*, 1997), which induces the production and secretion of MCP-1 and MCP-3 in K14-IL-17A$^{ind/+}$ skin, further propagating the inflammation. Apoptosis of neutrophils within K14-IL-17A$^{ind/+}$ skin is also likely to be a major contributor to soluble IL-6 receptor concentration in the skin (Chalaris *et al.*, 2007), but further experiments would be needed to confirm this. IL-6 has also been postulated to preside over a shift in inflammatory cell attraction by acting as a switch factor between neutrophil and monocyte recruitment (Kaplanski *et al.*, 2003). Although this is a possibility in the case of a bacterial infection, where the original insult will ultimately be cleared by the concerted efforts of the immune response, our model represents a chronic inflammatory state, where the otherwise T-cell–derived IL-17A is constitutively expressed by keratinocytes. The switch to monocyte recruitment will indeed take place, but the neutrophil chemotaxis will also continue because of the relentless release of IL-17A.

Taken together, our system recapitulates many hallmark features of human psoriasis and offers potential to examine the link between IL-17A-driven inflammation and the subsequent role of IL-6 aspects of neutrophil microabscess and psoriasiform lesion formation. Furthermore, given that IL-17A neutralization has already proven effective in the clinic, the K14-IL-17A$^{ind/+}$ system offers previously unreported perspectives on studying IL-17A-mediated skin inflammation in the mouse.

MATERIALS AND METHODS

Mice and scoring of psoriatic lesions

K14-IL-17A$^{ind/+}$ mice were generated by crossing the IL-17A^{ind} allele (previously described in Haak *et al.*, 2009) to the K14-Cre allele (Hafner *et al.*, 2004). Skin lesions were scored using a modified score based on the human psoriasis area and severity index score describing the degree of erythema, scaling of skin and skin thickness (0 = no affection, 1 = mild, 2 = intermediate, 3 = severe, 4 = very severe; El Malki *et al.*, 2013) and the percentage of affected skin referred to the total body surface. For the cumulative score, we multiplied the sum of the first three parameters with the percentage of the affected skin. Skin and epidermal thickness were measured in duplicates by using a micrometer (Mitutoyo, Aurora, IL). All animals were housed and used in experiments in accordance with institutional guidelines of the Central Animal Facility of the University of Mainz, Mainz, Germany.

In vitro assays and cytokine detection

Samples of skin of either IL-17A$^{ind/+}$ or K14-IL-17A$^{ind/+}$ mice were collected. Mononuclear cells were isolated from skin using liberase (Roche, Basel, Switzerland) and mechanical disruption. Skin-isolated

cells were cultured in RPMI-1640 containing 10% fetal calf serum, sodium pyruvate, non-essential amino acids and L-glutamine (all from Gibco, Deisenhofen, Germany). Cytokine release was measured in isolated supernatants using either a by either flow cytomix multiplex (BenderMedsystems, Salzburg, Austria) or ELISA (BD, Franklin Lake, NJ) after the common protocols.

Antibodies and flow cytometry

Lymph node cells, splenocytes, and skin-isolated cells were surface stained with anti-CD4, CD8, B220, GR1, CD11b, Ly6G, and IL-6Rα from BD. Further antibodies used include CD62L (Immunotools, Friesoythe, Germany). All antibodies were coupled to FITC, phycoerythrin, phycoerythrin-Cy5.5, phycoerythrin-Cy7, or antigen-presenting cell. Flow cytometric acquisition was performed on a FACScalibur (BD), Facs Canto II (BD) or LSR-Fortessa (BD) and analyzed with FlowJo software (Ashland, OR).

Real-time expression analysis and measurement

Total RNA from skin of K14-IL-17 $A^{ind/+}$ mice was isolated using the Trizol (Invitrogen, Karlsruhe, Germany). The expression of mRNA coding for T-bet, RORγt, IL-23-p19, Foxp3, and IL-6 were analyzed with specific primers from Qiagen as described on their homepage (https://www1.qiagen.com/GeneGlobe/Default.aspx) using the QuantiTect SYBR Green RT-PCR Kit. All changes in gene expression were calculated relative to that of glyceraldehyde-3-phosphate dehydrogenase or hypoxanthine-guanine phosphoribosyltransferase.

Histology

Skin samples were isolated from the back of experimental mice. Samples were fixed in 4% paraformaldehyde in phosphate-buffered saline, paraffin embedded, cut and stained with hematoxylin and eosin according to standard protocols.

Statistical analysis

Statistical significance was determined using the unpaired Student's *t*-test. Results are expressed as mean ± SEM.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Aggarwal S, Ghilardi N, Xie MH *et al.* (2003) Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J Biol Chem* 278:1910–4

Albanesi C, Scarponi C, Cavani A *et al.* (2000) Interleukin-17 is produced by both Th1 and Th2 lymphocytes, and modulates interferon-gamma- and interleukin-4-induced activation of human keratinocytes. *J Invest Dermatol* 115:81–7

Arican O, Aral M, Sasmaz S *et al.* (2005) Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. *Mediators inflammation* 2005:273–9

Aujla SJ, Dubin PJ, Kolls JK (2007) Interleukin-17 in pulmonary host defense. *Exp Lung Res* 33:507–18

Bartoccioni E, Scuderi F, Marino M *et al.* (2003) IL-6, monocyte infiltration and parenchymal cells. *Trends Immunol* 24:299–300. author reply 300-291

Becher B, Pantelyushin S (2012) Hiding under the skin: interleukin-17-producing gammadelta T cells go under the skin? *Nat Med* 18:1748–50

Castells-Rodellas A, Castell JV, Ramirez-Bosca A *et al.* (1992) Interleukin-6 in normal skin and psoriasis. *Acta Derm Venereol* 72:165–8

Chalaris A, Rabe B, Paliga K *et al.* (2007) Apoptosis is a natural stimulus of IL6R shedding and contributes to the proinflammatory trans-signaling function of neutrophils. *Blood* 110:1748–55

Cho JS, Pietras EM, Garcia NC *et al.* (2010) IL-17 is essential for host defense against cutaneous Staphylococcus aureus infection in mice. *J Clin Invest* 120:1762–73

Di Cesare A, Di Meglio P, Nestle FO (2009) The IL-23/Th17 axis in the immunopathogenesis of psoriasis. *J Invest Dermatol* 129:1339–50

El Malki K, Karbach SH, Huppert J *et al.* (2013) An alternative pathway of imiquimod-induced psoriasis-like skin inflammation in the absence of interleukin-17 receptor a signaling. *J Invest Dermatol* 133:441–51

Ellinghaus E, Ellinghaus D, Stuart PE *et al.* (2010) Genome-wide association study identifies a psoriasis susceptibility locus at TRAF3IP2. *Nat Genet* 42:991–5

Farley E, Menter A (2011) Psoriasis: comorbidities and associations. *G Ital Dermatol Venereol* 146:9–15

Fujishima S, Watanabe H, Kawaguchi M *et al.* (2010) Involvement of IL-17F via the induction of IL-6 in psoriasis. *Arch Dermatol Res* 302:499–505

Girolomoni G, Mrowietz U, Paul C (2012) Psoriasis: rationale for targeting interleukin-17. *Br J Dermatol* 167:717–24

Greaves MW, Weinstein GD (1995) Treatment of psoriasis. *N Engl J Med* 332:581–8

Grossman RM, Krueger J, Yourish D *et al.* (1989) Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. *Proc Natl Acad Sci USA* 86:6367–71

Gudjonsson JE, Johnston A, Dyson M *et al.* (2007) Mouse models of psoriasis. *J Invest Dermatol* 127:1292–308

Haak S, Croxford AL, Kreymborg K *et al.* (2009) IL-17A and IL-17F do not contribute vitally to autoimmune neuro-inflammation in mice. *J Clin Invest* 119:61–9

Hafner M, Wenk J, Nenci A *et al.* (2004) Keratin 14 Cre transgenic mice authenticate keratin 14 as an oocyte-expressed protein. *Genesis* 38:176–81

Harper EG, Guo C, Rizzo H *et al.* (2009) Th17 cytokines stimulate CCL20 expression in keratinocytes in vitro and in vivo: implications for psoriasis pathogenesis. *J Invest Dermatol* 129:2175–83

Harrison OJ, Foley J, Bolognese BJ *et al.* (2008) Airway infiltration of CD4+ CCR6+ Th17 type cells associated with chronic cigarette smoke induced airspace enlargement. *Immunol Lett* 121:13–21

Hedrick MN, Lonsdorf AS, Shirakawa AK *et al.* (2009) CCR6 is required for IL-23-induced psoriasis-like inflammation in mice. *J Clin Invest* 119:2317–29

Hirota K, Yoshitomi H, Hashimoto M *et al.* (2007) Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. *J Exp Med* 204:2803–12

Hoeve MA, Savage ND, de Boer T *et al.* (2006) Divergent effects of IL-12 and IL-23 on the production of IL-17 by human T cells. *Eur J Immunol* 36:661–70

Hoge J, Yan I, Janner N *et al.* (2013) IL-6 controls the innate immune response against Listeria monocytogenes via classical IL-6 signaling. *J Immunol* 190:703–11

Kaplanski G, Marin V, Montero-Julian F *et al.* (2003) IL-6: a regulator of the transition from neutrophil to monocyte recruitment during inflammation. *Trends Immunol* 24:25–9

Kelly MN, Kolls JK, Happel K *et al.* (2005) Interleukin-17/interleukin-17 receptor-mediated signaling is important for generation of an optimal polymorphonuclear response against Toxoplasma gondii infection. *Infect Immun* 73:617–21

Kishimoto T, Hibi M, Murakami M *et al.* (1992) The molecular biology of interleukin 6 and its receptor. *Ciba Found Symp* 167:5–16; discussion 16-23

Koga C, Kabashima K, Shiraishi N *et al.* (2008) Possible pathogenic role of Th17 cells for atopic dermatitis. *J Invest Dermatol* 128:2625–30

Kolls JK, Linden A (2004) Interleukin-17 family members and inflammation. *Immunity* 21:467–76

Lambert JR, Wright V (1976) Eye inflammation in psoriatic arthritis. *Ann Rheum Dis* 35:354–6

Lillard JW Jr, Singh UP, Boyaka PN *et al.* (2003) MIP-1alpha and MIP-1beta differentially mediate mucosal and systemic adaptive immunity. *Blood* 101:807–14

Lowes MA, Kikuchi T, Fuentes-Duculan J *et al.* (2008) Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *J Invest Dermatol* 128:1207–11

Maini RN, Taylor PC, Szechinski J *et al.* (2006) Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. *Arthritis Rheum* 54:2817–29

McLoughlin RM, Witowski J, Robson RL *et al.* (2003) Interplay between IFN-gamma and IL-6 signaling governs neutrophil trafficking and apoptosis during acute inflammation. *J Clin Invest* 112:598–607

Nestle FO, Conra C (2004) The IL-12 family member p40 chain as a master switch and novel therapeutic target in psoriasis. *J Invest Dermatol* 123: xiv–xv

Neurath MF, Finotto S (2011) IL-6 signaling in autoimmunity, chronic inflammation and inflammation-associated cancer. *Cytokine Growth Factor Rev* 22:83–9

Nograles KE, Brasington RD, Bowcock AM (2009) New insights into the pathogenesis and genetics of psoriatic arthritis. *Nat Clin Pract Rheumatol* 5:83–91

Ogura H, Murakami M, Okuyama Y *et al.* (2008) Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. *Immunity* 29:628–36

Pantelyushin S, Haak S, Ingold B *et al.* (2012) Rorgammat + innate lymphocytes and gammadelta T cells initiate psoriasiform plaque formation in mice. *J Clin Invest* 122:2252–6

Patel DD, Lee DM, Kolbinger F *et al.* (2013) Effect of IL-17A blockade with secukinumab in autoimmune diseases. *Ann Rheum Dis* 72 Suppl 2:ii116–23

Rincon M. (2012) Interleukin-6: from an inflammatory marker to a target for inflammatory diseases. *Trends Immunol* 33:571–7

Romano M, Sironi M, Toniatti C *et al.* (1997) Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity* 6:315–25

Sano S, Chan KS, Carbajal S *et al.* (2005) Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. *Nat Med* 11:43–9

Schall TJ, Bacon K, Camp RD *et al.* (1993) Human macrophage inflammatory protein alpha (MIP-1 alpha) and MIP-1 beta chemokines attract distinct populations of lymphocytes. *J Exp Med* 177:1821–6

Shear NH, Prinz J, Papp K *et al.* (2008) Targeting the interleukin-12/23 cytokine family in the treatment of psoriatic disease. *J Cutan Med Surg* 12(Suppl 1):S1–10

Taub DD, Conlon K, Lloyd AR *et al.* (1993) Preferential migration of activated CD4 + and CD8 + T cells in response to MIP-1 alpha and MIP-1 beta. *Science* 260:355–8

Teunissen MB, Koomen CW, de Waal Malefyt R *et al.* (1998) Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. *J Invest Dermatol* 111:645–9

Toruniowa B, Krasowska D, Koziol M *et al.* (1995) Serum levels of IL-6 in mycosis fungoides, psoriasis, and lichen planus. *Ann N Y Acad Sci* 762:432–4

Turner JE, Paust HJ, Steinmetz OM *et al.* (2010) CCR6 recruits regulatory T cells and Th17 cells to the kidney in glomerulonephritis. *J Am Soc Nephrol* 21:974–85

van der Fits L, Mourits S, Voerman JS *et al.* (2009) Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol* 182:5836–45

Waisman A (2012) To be 17 again–anti-interleukin-17 treatment for psoriasis. *N Engl J Med* 366:1251–2

Walter A, Schafer M, Cecconi V *et al.* (2013) Aldara activates TLR7-independent immune defence. *Nat Commun* 4:1560

Wang C, Kang SG, Lee J *et al.* (2009) The roles of CCR6 in migration of Th17 cells and regulation of effector T-cell balance in the gut. *Mucosal Immunol* 2:173–83

Zenz R, Eferl R, Kenner L *et al.* (2005) Psoriasis-like skin disease and arthritis caused by inducible epidermal deletion of Jun proteins. *Nature* 437:369–75

Zhou X, Chen Q, Moore J *et al.* (2009) Critical role of the interleukin-17/interleukin-17 receptor axis in regulating host susceptibility to respiratory infection with Chlamydia species. *Infect Immun* 77:5059–70