Distinct Roles of IL-23 and IL-17 in the Development of Psoriasis-Like Lesions in a Mouse Model

Kimiko Nakajima, Takashi Kanda, Mikiro Takaishi, Takeo Shiga, Ken Miyoshi, Hideki Nakajima, Reiko Kamijima, Masahito Tarutani, Jacqueline M. Benson, M. Merle Elloso, Lester L. Gutshall, Michael F. Naso, Yoichiro Iwakura, John DiGiovanni and Shigetoshi Sano

J Immunol published online 23 February 2011
http://www.jimmunol.org/content/early/2011/02/23/jimmunol.1000148

Supplementary Material http://www.jimmunol.org/content/suppl/2011/02/23/jimmunol.1000148.DC1

Subscription Information about subscribing to The Journal of Immunology is online at: http://jimmunol.org/subscription

Permissions Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts

Errata An erratum has been published regarding this article. Please see next page or: /content/187/11/6157.full.pdf
Distinct Roles of IL-23 and IL-17 in the Development of Psoriasis-Like Lesions in a Mouse Model

Kimiko Nakajima,* Takashi Kanda,* Mikio Takaishi,* Takeo Shiga,* Ken Miyoshi,* Hideki Nakajima,* Reiko Kamijima,* Masahito Tarutani,* Jacqueline M. Benson,† M. Merle Elloso,‡ Lester L. Gutshall,‡ Michael F. Nabo,‡ Yoichiro Ikawura,‡ John DiGiovanni,§& and Shigetoshi Sano*†

Psoriasis is an inflammatory disease with dynamic interactions between the immune system and the skin. The IL-23/Th17 axis plays an important role in the pathogenesis of psoriasis, although the exact contributions of IL-23 and IL-17 in vivo remain unclear. K5.Stat3C transgenic mice constitutively express activated Stat3 within keratinocytes, and these animals develop skin lesions with histological and cytokine profiles similar to those of human plaque psoriasis. In this study, we characterized the effects of anti-mouse IL-17A, anti-mouse IL-12/23p40, and anti-mouse IL-23p19 Abs on the development of psoriasis-like lesions in K5.Stat3C transgenic mice. Treatment with anti–IL-12/23p40 or anti–IL-23p19 Abs greatly inhibited 12-O-tetradecanoylphorbol-13-acetate–induced epidermal hyperplasia in the ears of K5.Stat3C mice, whereas the inhibitory effect of an anti–IL-17A Ab was relatively less prominent. Treatment with anti–IL-12/23p40 or anti–IL-23p19 Abs markedly lowered transcript levels of Th17 cytokines (e.g., IL-17 and IL-22), β-defensins, and S100A family members in skin lesions. However, anti–IL-17A Ab treatment did not affect mRNA levels of Th17 cytokines. Crossing IL-17A–deficient mice with K5.Stat3C mice resulted in partial attenuation of 12-O-tetradecanoylphorbol-13-acetate–induced lesions, which were further attenuated by anti–IL-12/23p40 Ab treatment. FACS analysis of skin-draining lymph node cells from mice that were intradermally injected with IL-23 revealed an increase in both IL-22–producing T cells and NK-22 cells. Taken together, this system provides a useful mouse model for psoriasis and demonstrates distinct roles for IL-23 and IL-17.

Psoriasis is one of the most common inflammatory skin disorders and affects ≥2% of the population in Western countries. Histologically, psoriasis is characterized by epidermal hyperplasia (acanthosis), dermal infiltration of immune cells, and hyperkeratosis. Psoriatic pathological features also include excessive proliferation and impaired differentiation of epidermal keratinocytes, likely mediated by a dysregulated immune system (1–3). Our previous studies elucidated the role of Stat3 signaling in keratinocytes during the development of psoriatic lesions (4–6). For example, K5.Stat3C transgenic mice, in which Stat3 is constitutively expressed in keratinocytes, developed psoriasiform lesions following wounding stimuli or topical treatment with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA).

Recent studies have demonstrated that the IL-23/Th17 pathway is linked to a number of inflammatory diseases, including psoriasis and animal models of multiple sclerosis, arthritis, and inflammatory bowel disease (7–11). The most distinct evidence for the role of IL-23/Th17 in psoriasis comes from clinical studies. Recent clinical trials showed that therapy with an anti–IL-12/IL-23p40 Ab (ustekinumab) is an effective treatment for psoriasis (12–14). TNF-α inhibitors are also widely used to treat autoimmune diseases, including psoriasis, and they significantly improve psoriasis area severity index scores. Notably, inhibition of TNF by a soluble TNF-α receptor antagonist (etanercept) was associated with reduced Th17 responses (15). Because Th1 responses were not affected, that study suggests that Th17 cells are particularly important in driving psoriasis. Therefore, amelioration of psoriasis has been associated with reduced Th17 responses (15, 16). IL-22, another cytokine produced by Th17 cells, is elevated in the blood of psoriasis patients (17). Triggering the IL-22 receptor induces proliferation and migration of keratinocytes, but reduces their differentiation (18). Furthermore, studies using either a mouse model for psoriasis or reconstituted human epidermis revealed that IL-22 mediates acanthosis through the activation of Stat3 in keratinocytes. We have recently demonstrated that topical application of a small molecule Stat3 inhibitor resulted in clinical amelioration of psoriatic lesions, indicating that Stat3 signaling in the epidermis is essential for psoriasis development (6). Collectively, these observations strongly suggest that IL-22 mediates crosstalk between immunocytes and keratinocytes in the pathogenesis of psoriasis (19–21).

Previous studies have demonstrated that IL-17 and IL-22 cooperatively enhance gene expression of antimicrobial peptides by keratinocytes including β-defensin 2, 3, and S100A7/8/9, all of which are also up-regulated in psoriatic lesions (18, 22). IL-17...
alone has been shown to induce expression of chemokines for neutrophils, memory T cells, and dendritic cells (23), suggesting that IL-17 is proinflammatory. Whereas, IL-22 facilitates keratinocyte proliferation and retards differentiation (18, 20). Initial observations from a phase I clinical trial showed that the signs and symptoms of rheumatoid arthritis are significantly suppressed following treatment with an anti–IL-17 Ab, without notable adverse effects (24). In addition, IL-17 blockade has been suggested to have therapeutic effectiveness on psoriasis (25). Whether the roles of IL-17 and IL-22 can be dissected in the pathogenesis of psoriasis remains largely unknown.

In this study, we investigated the therapeutic effects of anti–IL-12/23, anti–IL-23, and anti–IL-17 Abs on the development of skin lesions in K5.Stat3C mice and compared changes in gene expression of cytokines following Ab treatment. The skin lesions of K5.Stat3C mice resemble human psoriasis based on clinical, histological, immunophenotypic, and biochemical criteria used to evaluate animal models of psoriasis (26). TPA-induced psoriasis-like lesions were sensitive to treatment with anti–IL-12/23 and anti–IL-23 Abs. In contrast, anti–IL-17A Ab treatment was less effective than anti–IL-12/23 or anti–IL-23. These data suggest that K5.Stat3C mice meet pharmacological criteria and represent one of the most physiologically relevant animal models for psoriasis. Furthermore, our mechanistic studies suggest that IL-23 regulates not only T cells but also NK cell populations in this model of human psoriasis.

Materials and Methods

Patients with psoriasis

The study protocol was conducted in accordance with the guidelines of the World Medical Association’s Declaration of Helsinki and was approved by the Institute Ethical Review Board of the Kochi Medical School, Kochi University. Eight patients with chronic plaque-type psoriasis (five males, three females) were recruited from the Department of Dermatology, Kochi Medical School Hospital in Nankoku, Kochi, Japan. Skin biopsy specimens were taken from lesions and from contiguous uninvolved skin of patients with psoriasis.

RNA isolation and real-time RT-PCR

Skin biopsy specimens from either psoriasis patients or mice were minced with scissors into small pieces on ice, then disrupted by ultrasonic sonication in RNA lysis buffer (RNeasy Mini Kit, Qiagen). Total RNA was extracted using an RNA isolation kit (Promega) according to the manufacturer’s protocol and was reverse transcribed using M-MLV reverse transcriptase (Invitrogen) with random oligonucleotide hexamers (Invitrogen). In some experiments, primary cultured mouse keratinocytes and lymph node cells were subjected to RNA isolation and RT-PCR. PCR reactions were performed using Power SYBER Green PCR Master Mix (Applied Biosystems), and amplification conditions were as follows: 50°C for 2 min, 90°C for 10 min for 1 cycle, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The primers used are listed in Supplemental Data. The quantity of each transcript was analyzed using the 7000 Fast System Software (Applied Biosystems) and was normalized to housekeeping genes, such as IL-12/23p40, IL-23p19, IL-17A, IL-17F, and IL-22, and axis genes, such as IL-12/23p40, IL-23p19, IL-17A, IL-17F, and IL-22, and axis genes, such as IL-12/23p40, IL-23p19, IL-17A, IL-17F, and IL-22, and axis genes, such as IL-12/23p40, IL-23p19, IL-17A, IL-17F, and IL-22, and axis genes, such as IL-12/23p40, IL-23p19, IL-17A, IL-17F, and IL-22, and axis genes, such as IL-12/23p40, IL-23p19, IL-17A, IL-17F, and IL-22, and axis genes, such as IL-12/23p40, IL-23p19, IL-17A, IL-17F, and IL-22.

K5.Stat3C transgenic mice, early TPA responses in the ear, induction of bona fide psoriasiform lesions, and administration of Abs

All experimental procedures performed on mice were approved by the Institutional Animal Care and Use Committee of the Kochi Medical School. K5.Stat3C mice were generated as previously reported, and heterozygous K5.Stat3C mice were backcrossed with IL-17A−/−:K5.Stat3C mice. The littermates of IL-17A−/−:K5.Stat3C mice were used as controls for IL-17A−/−:K5.Stat3C mice.

Neutralizing Abs

Blocking Abs included rat/mouse chimeric anti-mouse IL-17A (CNTO 8096), anti-mouse IL-12/23p40 (CNTO 3913), and anti-mouse IL-23p19 (CNTO 6163). As a control Ab, rat/mouse chimeric mouse IgG control (CNTO 1322) was used. CNTO 3913, CNTO 6163, and CNTO 1322 were provided by Centocor Research & Development (Radnor, PA) and have been previously described (28, 29). For CNTO8096 generation, cDNA from a rat anti-mouse IL-17A hybridoma clone was generated using the 5′ RACE Generacer Kit (Invitrogen). H and L chain variable regions were amplified using the Generacer 5′ primer along with primer 644 [rat κ-5′:CTCTAGCTGTAGGTGCTGTC-3] or primer 641 [rat IgG2a-5′:TG-GGCAACUTTGCAAGTGAC-3′(Hc)]. The V region genes were amplified and cloned into expression vectors containing mouse IgG2a Hc and mouse κ sequences, using the ligase-independent cloning method. A double gene vector, containing the full coding sequence of both H and L chains, was assembled by restriction enzyme digestion and ligation and used to transfect CHO-K1 cells for stable cell line development. Expressed Ab was batch protein A purified from the stable cell culture supernatant. Neutralization activity of CNTO 8096 was demonstrated in vitro via inhibition of IL-17A–induced keratinocyte-derived chemokine production from NIH3T3 cells (data not shown).

FIGURE 1. Profiles of cytokines, β-defensins, and S100A family proteins in human psoriatic lesional skin relative to contiguous nonlesional skin. Real-time RT-PCR reveals increased mRNA levels of the IL-23/Th17 axis genes, such as IL-12/23p40, IL-23p19, IL-17A, IL-17F, and IL-22, and β-defensins (hBD) and S100A family proteins. Gene expression levels were normalized to transcripts of the HPRT gene. The transcriptional level of each gene in lesional skin was plotted compared with nonlesional skin (set to 1). Bars indicate geometric mean values, n = 8.
**Immunohistochemical staining**

For detection of Th17 cells in ear skins that were topically treated with TPA, snap-frozen sections were treated with a blocking reagent (Protein Block Serum-Free; Dako) for an hour at room temperature, treated with rat anti-mouse CD4 mAb (H129.19; BD Pharmingen), and rabbit anti-mouse IL-17A polyclonal Ab (H-132; Santa Cruz Biotechnology) for overnight at 4°C, followed by treatment with anti-rat IgG-Alexa 488, and anti-rabbit IgG-Alexa 594 (Invitrogen), respectively. In some experiments, anti-mouse CD6 mAb (53-6-7; BD Pharmingen) was used. For staining with anti-K6/7 mAb (Thermo Scientific), formalin-fixed skin specimens were deparaffinized, incubated in 10 mM citrate buffer (pH 6.0), and autoclaved.

**FACS analysis**

For draining lymph node cell analyses, cervical lymph nodes were collected from K5.Stat3C mice with ear skin lesions induced by TPA treatment or intradermal injection of 400 ng IL-23 (R&D Systems) for 5 consecutive days. Cells were stimulated for 4 h with 0.2 μg ml⁻¹ PMA (Wako Chemicals), 2.7 μM ionomycin (Sigma-Aldrich), and 40 μg ml⁻¹ brefeldin A (Sigma-Aldrich). Then cells were stained for 20 min with FITC (BioLegend)- or Cy5-conjugated anti-CD3 mouse Ab (BD Pharmingen), followed by permeabilization with Cytofix/Cytoperm buffer (BD Pharmingen) and intracellular staining with PE-conjugated anti–IL-22 mouse Ab (R&D Systems). In some experiments, cells were stained with FITC-conjugated anti-CD56 Ab (BioLegend). Samples were acquired on a FACS Calibur flow cytometer, and data analysis was conducted using CellQuest Pro software (BD Biosciences).

**Cell sorting**

Skin lesion-draining lymph node cells were labeled with anti-mouse CD3ε Ab conjugated to biotin and treated with microbeads conjugated to monochromic anti-biotin Ab (Miltenyi Biotec). Cells were then separated into CD3-positive or -negative cells by MACS using a magnetic column, according to the manufacturer’s instructions (Miltenyi Biotec). The purity of sorted CD3⁺ T cells was >98% (data not shown).

**Statistical analysis**

Statistical analysis of significance was calculated using the Mann–Whitney U test, the Student t test, or the Kruskal–Wallis one-way analysis. A p value < 0.05 was considered significant, and all data are shown as mean ± SD.

**Results**

Changes in cytokine profiles of human psoriatic lesions

To investigate cytokine profiles of human psoriatic lesions, we compared transcriptional levels of cytokines from lesions with those from contiguous uninvolved skin, using real-time RT-PCR. Similar to previous reports, psoriatic lesions showed increased transcriptional levels of the IL-23/Th17 axis, including IL-23p19, IL-12/23p40, IL-17A, IL-17F, and IL-22, whereas IL-12p35 and IL-4 were not increased (Fig. 1). p40 is the common subunit of IL-12 and IL-23, whereas p19 and p35 are distinct subunits of IL-23 and IL-12, respectively. The present result is consistent with previous reports that IL-23 mRNA expression is greater than IL-12 mRNA in human psoriasis (30). β-defensin2, β-defensin3, and S100A family members, such as S100A7 (psoriasin), S100A8, and S100A9, were also markedly upregulated, as previously reported (20). Considering the data together, we verified that psoriatic lesions showed increased levels of mRNAs encoding keratinocyte microbial peptides and members of the IL-23/Th17 axis.

**Topical TPA-induced lesions in K5.Stat3C transgenic mice contain dermal Th17 infiltrates**

K5.Stat3C mice constitutively express activated Stat3 in keratinocytes and epidermal hyperplasia upon stimulation with TPA topical treatment (4, 5, 31). Following two topical treatments with...
TPA on days 1 and 3, K5.Stat3C mice, but not wild-type mice, demonstrated a marked epidermal hyperplasia in the ear on day 4 (Fig. 2A). Immunohistochemical examination revealed increased Th17 cell infiltrates (CD4+IL-17+ cells) in the dermis of TPA-induced lesions in K5.Stat3C mice (Fig. 2B). In addition, CD4+ IL-17+ cells and Tc17 cells were present (CD8+IL-17+; data not shown). This result suggested that similar to human psoriatic lesions, Th17 cell infiltrates might contribute to epidermal hyperplasia in K5.Stat3C mice.

Cytokine profiles of K5.Stat3C mouse skin lesions are similar to those of human psoriatic lesions

Real-time RT-PCR analysis revealed that short-term TPA treatment-induced skin lesions in K5.Stat3C mice showed increased transcript levels of Th17 cytokines, including IL-17A, IL-17F, and IL-22. IL-23p19 and IL-12/23p40 were upregulated, but IL-12p35 was marginally affected (Fig. 2C). Some proinflammatory cytokines, including TNF-α, IL-1β, and IL-6, were increased. However, other cytokines, such as IFN-γ and TGF-β, were not. It should also be noted that β-defensin (hBD) 3, 4 and S100A8, 9 were upregulated. Collectively, these data indicated that TPA-induced lesions in K5.Stat3C mice demonstrate a cytokine and antimicrobial molecule profile that generally resembles that found in human psoriatic lesions (Fig. 1).

Effects of Abs against IL-12/23p40, IL-23p19, and IL-17A on the generation of TPA-induced epidermal hyperplasia in K5.Stat3C mice

It has been well recognized that anti–IL-12/23p40 Ab treatment can attenuate human psoriasis (12–14). It has been suggested that anti–IL-12/23p40 Ab treatment is effective in treating psoriasis, but the mechanism of its action is not fully understood. In this study, we investigated the efficacy of Abs against IL-12/23p40, IL-23p19, and IL-17A in the generation of TPA-induced epidermal hyperplasia in K5.Stat3C mice. Administration of anti–IL-17A Ab 3 d prior to the first treatment with TPA partially attenuated epidermal hyperplasia (Fig. 3A). Administration of anti–IL-12/23p40 Ab as well as by anti–IL-12/23p19 Ab (Fig. 3A, 3B), when compared with control IgG-treated mice. Immunohistochemical staining with anti-Ki67 revealed that anti–IL-17A significantly reduced epidermal proliferation. Keratinocyte numbers positive for anti-Ki67 were reduced by 23% (Fig. 3A, 3B). In contrast, epidermal hyperplasia was markedly attenuated by anti–IL-12/23p340 Ab as well as by anti–IL-12/23p19 Ab (Fig. 3A, 3B), when compared with control IgG-treated mice. Immunohistochemical staining with anti-Ki67 revealed that anti–IL-12/23p40 Ab treatment further abolished it (Fig. 3C). Because rIL-17A did not facilitate keratinocyte proliferation in culture (data not shown), administration of anti–IL-17A might indirectly impair keratinocyte proliferation through downregulation of chemokine expressions (Supplemental Fig. 1). Efficacy of anti–IL-12/23p40 and anti–IL-23p19 Abs suggested the dominant role of IL-23 in this model of psoriasis, but a small, if any, contribution of the IL-12/Th1 axis, which would also be sensitive to the anti–IL-12/23p40 Ab. This result also indicated that the skin response to TPA of K5.Stat3C mice was dependent on IL-23, but only partially dependent on IL-17A alone.

Decreased expression of psoriasis-associated genes following administration of anti–IL-12/IL-23p40 or anti–IL-23p19 Abs

To study differences in gene expression between mice that were treated with control IgG or anti–IL-12/23p40, anti–IL-23p19, or anti–IL-17A Abs, real-time RT-PCR was performed on lesional skin samples after short-term TPA treatment. Administration of anti–IL-12/23p40 or anti–IL-23p19 Abs equally lowered transcriptional levels of IL-17A, IL-17F, and IL-22, suggesting that IL-23 blockade with either Ab resulted in inhibition of the development of pathogenic Th17 cells (Fig. 4A). In contrast, administration of anti–IL-17A did not decrease but rather increased the transcription of IL-17A, IL-17F, and IL-22 genes, although not to a statistically significant level. Furthermore, anti–IL-12/23p40 or anti–IL-23p19 Ab treatment decreased expression of psoriasis-associated keratinocyte genes, including β-defensins and S100A family genes (Fig. 4A). In contrast, the inhibitory effect of anti–IL-17A treatment was not obvious. Similarly, administration of anti–IL-12/23p40 markedly reduced transcript levels of chemokines, including CXCL1, 2, 3, and 5, whereas anti–IL-17A treatment resulted in only a modest decrease (Supplemental Fig. 1). It is worth noting that treatment with anti–IL-17A significantly ameliorated formation of neutrophil-containing intraepidermal abscesses within TPA-induced lesions (Fig. 4C, Supplemental Fig. 2), indicating a role for IL-17A in neutrophil recruitment to lesions. Neutrophilic abscesses were not observed in lesions after treatment with anti–IL-12/23p40 or anti–IL-23p19 (data not shown).

As previously reported (23), in vitro stimulation of cultured epidermal keratinocytes from K5.Stat3C mice with recombinant IL-17A, IL-22, or both, revealed that IL-17A alone upregulated gene expression of chemokines, whereas IL-22 did not (Fig. 4B, top panel). In contrast, a synergistic effect of IL-17A plus IL-22 was demonstrated by upregulation of β-defensins and S100A families, although not to a statistically significant level (Fig. 4B, bottom panel). This result is in agreement with the previous...
studies (22, 23, 32) and suggests that although IL-17A is a cytokine induced by IL-23, IL-22 is another cytokine downstream of IL-23 that might be critical for development of psoriasis (Figs. 3, 4, Supplemental Fig. 1) (22).

Effect of anti–IL-12/23p40 or anti–IL-23p19 Abs on development of psoriasis-like skin lesions in K5.Stat3C mice

Repeated topical treatment with TPA for more than 1 mo induced bona fide psoriasiform lesions in K5.Stat3C mice, as previously reported (4). The lesions recapitulated human psoriasis with histological similarity, including acanthosis, elongation of rete ridges, hyperkeratosis, parakeratosis, proliferation of capillaries, mononuclear cell infiltrates in the dermis, and neutrophil infiltrates in the epidermis. Administration of either anti–IL-12/23p40 or anti–IL-23p19 Abs markedly inhibited the development of bona fide psoriasiform lesions in K5.Stat3C mice (Fig. 5A, 5B). The inhibitory effect of anti–IL-12/23p40 or anti–IL-23p19 Abs was more prominent than that by anti–IL-17A. This result again suggested that IL-23 played a predominant role over IL-17A alone in the development of psoriasis-like lesions in this model. Further, it was shown that the IL-23/Th17 axis outperformed the IL-12/Th1 axis, because the anti–IL-23p19 Ab, which is specific for IL-23, showed inhibitory effect comparable to that of anti–IL-12/23p40 Ab, which blocks both IL-12 and IL-23 signaling.

Effect of anti–IL-12/23p40 Ab on the generation of psoriasiform lesions in IL-17A−/−:K5.Stat3C mice

The importance of IL-17–producing T cells in the development of autoimmune diseases has been suggested by in vivo experiments with mice in which the IL-17A gene was ablated. For example, collagen-induced arthritis and experimental autoimmune encephalomyelitis did not develop in IL-17A−/− mice (33, 34). Thus, we asked whether this was also the case with TPA-induced psoriasis-like formation in K5.Stat3C mice. IL-17A−/−:K5.Stat3C mice developed mild psoriasis-like lesions compared with IL-17A+/−: K5.Stat3C mice, suggesting that IL-17A partially contributes to

FIGURE 4. A, Effect of Abs on transcriptional levels of Th17 cytokines, β-defensins, and S100As in ear skin of K5.Stat3C mice after short-term TPA treatment. Real-time RT-PCR of lesional skins revealed that anti–IL-12/23p40 (green bars) and anti–IL-23p19 (purple bars) Abs lowered transcriptional levels of Th17 cytokines, including IL-17A, IL-17F, and IL-22, β-defensin 3 (BD3), β-defensin 4 (BD4), S100A8, and S100A9, compared with the control IgG-treated group (blue bars), set to 1. Increases in Th17 cytokine mRNAs were elicited by anti–IL-17 Ab treatment (red bars) but were not statistically significant. IgG control (n = 16), anti–IL-17A (n = 8), anti–IL-12/23p40 (n = 14), and anti–IL-23p19 (n = 6). *p < 0.05, **p < 0.01, Kruskal–Wallis one-way analysis. B, Effects of IL-17A, IL-22, and their combination on gene expression of chemokines and antimicrobial peptides in primary epidermal keratinocytes of K5.Stat3C mice. Primary keratinocytes were stimulated in vitro with recombinant IL-17A (10 ng/ml), IL-22 (10 ng/ml), or both for 24 h, then subjected to real-time RT-PCR. Unstimulated transcripts normalized to HPRT were set to 1. Representative data are shown from three independent experiments. IL-17A alone (green bars) upregulated transcripts of CXCL1, -2, -3, and -5, and microbial peptides, although IL-22 (black bars) did not affect the gene expression tested. A small synergistic effect of IL-17A plus IL-22 (orange bars) on gene expression of S100As and β-defensins was observed. C, Anti–IL-17A treatment reduced formation of neutrophilic abscesses in TPA-induced lesions. The number of epidermal abscesses was counted per ear of K5. Stat3 mice treated with control IgG (blue bar, n = 4) or anti–IL-17A (red bar, n = 4), followed by short-term TPA treatment. Values were shown by mean ± SD. *p < 0.05, Student t test.
generated in therapeutic efficacy between Abs on psoriatic plaques. Increased IL-22–producing T cells and NK-22 cells in lesional skin-draining lymph nodes of K5.Stat3C mice

Because TPA-induced skin lesions of K5.Stat3C mice demonstrated upregulation of transcript levels of Th17 cytokines and accumulation of Th17 cells, we examined whether IL-22–producing cells were also increased in lesional skin-draining lymph node cells. FACS analysis revealed that IL-22–producing cells were increased not only in CD3+ but also in CD3− populations upon topical TPA treatment (Fig. 7A). Comparable IL-22 transcript levels were found in both CD3-enriched and CD3-depleted cells sorted from draining lymph node cells (Fig. 7B). According to FACS data, IL-22+ cells constituted 2.04% of all the CD3+ cells and 4.97% of all the CD3− cells. Therefore, the frequency of IL-22–producing cells in the lymph nodes was found more within non-T cells than within T cells, although the CD3−IL-22+ cells were present in greater numbers than the CD3+IL-22+ cells (Fig. 7A). It should be noted that IL-17A mRNA and IL-23p19 mRNA were exclusively present in CD3-enriched and −depleted cells, respectively (Fig. 7B). This result suggested that IL-22 was produced not only by T cells but also by non-T cells, whereas IL-23 was produced from non-T cells such as dendritic cells. Similar to topical TPA treatment, intradermal injection of IL-23 also resulted in formation of psoriasis-like lesions in the ears, as previously reported (19, 35). (Supplemental Fig. 3). IL-23 injection, like TPA treatment, increased CD3+ as well as CD3− IL-22–producing cells in lesional skin-draining lymph nodes (Fig. 7C). A striking observation was that staining with anti-CD56 revealed that, in the CD3− cell population, NK-22 cells (CD56−IL-22+) were increased by stimulation with IL-23, whereas CD56+IL-22+ cells remained unchanged. Actual numbers of CD3−IL-22–producing cells or NK-22 were increased in skin-draining lymph nodes upon topical TPA treatment (Fig. 7D). Taken collectively, the development of skin lesions in K5. Stat3C mice induced by IL-23 was associated with an increase in IL-22–producing T cells and NK-22 cells. This finding suggests that immune mechanisms triggered by topical treatment with TPA might include upregulation of IL-23 in the skin. In conclusion, we hypothesize that IL-23 is upstream of Th17, NK-22, and putative other cell lineages contributing to psoriasis development (Fig. 8).

Discussion

The pathogenesis of psoriasis is multifactorial, with genetic, environmental, and immunological factors contributing to the phenotype. The importance of T cells in the pathogenesis of psoriasis is supported by the response of patients to treatment with agents that affect T cell function, such as cyclosporine. Several cytokines have been implicated in the pathogenesis of psoriasis, including TNF-α, IFN-γ, IFN-α, IL-12, IL-17, and recently IL-23. Th17 cells are distinct from Th1 and Th2 cells in their differentiation and maintenance conditions, as well as in their effector cytokine expression profile. IL-23/Th17 signaling provides a link between the adaptive immune response and innate immunity, such as responses to bacterial infection and autoimmunity, including rheumatoid arthritis, experimental autoimmune encephalomyelitis, and psoriasis (7–11). The U.S. Food and Drug Administration has recently approved an anti-IL-12/23p40 Ab, named Stelara (ustekinumab), for treating moderate-to-severe plaque psoriasis (12–14). Although the p40 subunit is shared by IL-12 and IL-23, these two cytokines likely differ in their contribution to psoriasis. Accumulating evidence has linked IL-23 and Th17 cells to the patho-

FIGURE 5. Effect of Ab treatments on the development of bona fide TPA-induced psoriasiform lesions on the backs of K5.Stat3C mice. A, Representative macroscopic and histological views (H&E staining) of dorsal skins of mice treated with TPA following a single treatment with the indicated Abs. Scale bar, 200 μm. B, Epidermal thickness of the dorsal skin. Mean thickness ± SD (in micrometers). IgG control (n = 8), anti-IL-17A (n = 7), anti-IL-12/23p40 (n = 6), anti-IL-23p19 (n = 4). **p < 0.01, Mann–Whitney U test.

FIGURE 6. IL-17A gene deficiency attenuates epidermal hyperplasia of K5.Stat3C mice, but anti–IL-12/23p40 Ab treatment further abolishes the phenotype. A, Histological views of the dorsal skin of mice treated with TPA, as noted. Induced bona fide psoriasiform lesions were partially attenuated in IL-17A−/−:K5.Stat3C mice (middle left panel) compared with IL-17A+/−:K5.Stat3C mice (left panel), but anti–IL-12/23p40 Ab treatment abolished lesions in IL-17A−/−:K5.Stat3C mice (right panel), compared with those treated with control IgG (middle right panel). H&E staining. Scale bar, 100 μm. B, Epidermal thickness of the dorsal skins of mice, as indicated. Mean thickness ± SD (in micrometers). IL-17A+/−:K5.Stat3C (n = 8), IL-17A−/−:K5.Stat3C (n = 7), treated i.p. with control IgG (n = 4) or anti–IL-12/23p40 (n = 4). **p < 0.01, Mann–Whitney U test.
genesis of psoriasis (15, 16, 30, 36–39). Recent studies have demonstrated that polymorphisms in the IL-12/23p40 gene and IL-23R gene were associated with psoriasis (40–43). These findings suggested that genetic alteration of IL-23 signaling conferred increased risk of psoriasis. Further evidence for the role of IL-23 in the pathogenesis of psoriasis was supported by observations of psoriasis-like acanthosis upon repeated intradermal injections of IL-23 in mice (19, 35). The current study also demonstrates a role for IL-23 in psoriasis via comparable efficacy of Abs to IL-12/23R gene were associated with psoriasis (40–43). These findings suggested that genetic alteration of IL-23 signaling conferred increased risk of psoriasis. Further evidence for the role of IL-23 in the pathogenesis of psoriasis was supported by observations of psoriasis-like acanthosis upon repeated intradermal injections of IL-23 in mice (19, 35). The current study also demonstrates a role for IL-23 in psoriasis via comparable efficacy of Abs to IL-12/23p40 and IL-23p19 in the development of psoriasis-like lesions in the K5.Stat3C mouse model.

As we noted in this paper, human psoriatic skin has elevated IL-17 gene expression in lesions relative to uninvolved skin. However, the specific contribution of IL-17A to the pathogenesis of psoriasis is not well understood. Chan et al. (35) demonstrated that intradermal injection of IL-23 elevated IL-17A expression in mouse skin, but pretreatment with an anti–IL-17A Ab did not reduce psoriasiform lesion formation. This observation suggested that IL-17A was not required for IL-23–dependent epidermal hyperplasia. In the current study, a less dominant role for IL-17A in the development of skin lesions in K5.Stat3C mice was demonstrated through experiments with either administration of anti–IL-17A Ab or through IL-17A gene deficiency. IL-22 gene expression within skin lesions was not affected by anti–IL-17A, suggesting a limited effect of IL-17A on epidermal hyperplasia. Furthermore, only a moderate inhibition of epidermal proliferation by anti–IL-17A was observed, perhaps owing to incomplete downregulation of chemokine receptors. In other studies, neutrophil infiltrates were demonstrated to indirectly impact keratinocyte proliferation (44). As shown in this paper, pretreatment with anti–IL-17A decreased the number of neutrophilic abscesses in TPA-induced lesions. This result supports a role for IL-17A in recruitment of neutrophils. It is worth noting that IL-17F might compensate for the absence of IL-17A.

Our findings have also highlighted the importance of IL-22 in the pathogenesis of psoriasis. IL-22 plays an important role in maintaining homeostasis and remodeling of epithelial tissues, including epidermal keratinocytes, through regulating innate responses (45). Importantly, IL-22 mediates keratinocyte activation via phosphorylation of Stat3, leading to acanthosis that is associated with a psoriatic phenotype (18, 19). As such, Th22 cells, like Th17 cells, might also contribute to psoriasis pathology (46, 47). The present study in K5.Stat3C mice suggested that IL-22 contributed to the generation of skin lesions following topical TPA treatment or intradermal injection with IL-23. In the lesional skin-draining lymph nodes, IL-22–producing T cells and NK-22 cells were increased. However, we did not dissect the role of Th17 from Th22 cells in the current study. Therefore, the role of Th22 cells in our experimental settings remains to be determined. Recent studies have demonstrated that human NK-22 cells also have a part in mucosal immunity (48–50) and that IL-22 can be secreted in response to IL-23 (51). Further evaluation is required to determine if NK-22 cells are involved in human psoriasis.

IL-20 subfamily cytokines, including IL-20, IL-19, IL-22, IL-24, and IL-26, are involved in psoriasis, perhaps through stimulation of Stat3 activation of epidermal keratinocytes (20). Our previous study demonstrated that Stat3 activation is seen in psoriatic keratinocytes and that inhibition of Stat3 signaling using a specific decoy oligonucleotide or small molecule Stat3 inhibitor, STA-21, ameliorated psoriasis-like lesions of K5.Stat3C mice (4, 6). Our recent study also demonstrated that ointment containing 0.2% STA-21 improved psoriasis in a clinical feasibility study (6).
To explore psoriasis pathomechanisms and to test pharmacological efficacy, a number of animal models of psoriasis have been established (52). Proposed criteria to evaluate animal models of psoriasis include clinical, histological, immunophenotypic, biochemical, and pharmacological criteria (26). Strikingly, K5.Stat3C mice meet all these criteria as well as lesional gene expression criteria, demonstrating transcriptional similarity to human psoriatic lesions. Therefore, results shown in the current study with K5.Stat3C mice could be relevant to future research on human psoriasis.

In conclusion, the current study using K5.Stat3C mice demonstrates the pivotal role of IL-12/23p40 and IL-23p19 in this model of human psoriasis, with less contribution of IL-17A alone. As shown in Fig. 8, we hypothesize that IL-23 is the relevant upstream cytokine that is required for proliferation or maturation of Th17, NK-22, and other cells, to generate a psoriatic phenotype of the epidermis.

Acknowledgments
We thank W. Ouyang for critical suggestions.

Disclosures

References

8 DISTINCT ROLES OF IL-23 AND IL-17 IN PSORIASIS

TNF inhibition is associated with reduced Th17 responses. J. Exp. Med. 204: 3183–3194.


Corrections


After the publication of this article, we were informed that a reagent used in the studies was inadvertently provided in error.

The anti-mouse IL-23p19 Ab (CNTO 6163) provided to us by Centocor was subsequently determined to actually be CNTO 3913, an Ab to mouse IL-12/23p40, which neutralizes both IL-12 and IL-23. CNTO 3913 was also intentionally utilized in the studies and appropriately noted as an anti–IL-12/23p40 Ab. We included IL-23p19 Ab results in Figs. 3, 4, and 5. Not unexpectedly, we did not find significant differences between anti–IL-23p19 and anti–IL-12/23p40 effects. However, we did state conclusions within the text that designated the pharmacological effects to IL-23. This should be corrected to more accurately describe the effects as IL-12/23p40 mediated.

To address the Ab identity error, we have reproduced the studies with the proper CNTO 6163 Ab and provide here updated Figs. 3, 4, and 5. We believe that the main conclusions of the article remain true because there were significant differences between IL-23p19 and IL-17A inhibition.

We deeply regret this inadvertent error and apologize for any inconvenience this may have caused.

FIGURE 3. Effect of anti–IL-17A, anti–IL-12/23p40, or anti–IL-23p19 on early TPA responses in K5.Stat3C mice. A, Representative histological features of TPA-treated ear skin following treatment with the indicated Abs. H&E staining. Scale bar, 100 μm. B, Epidermal ear thickness. Mean thickness ± SD (in micrometers). IgG control (n = 8), anti–IL-17A (n = 4), anti–IL-12/23p40 (n = 6), anti–IL-23p19 (n = 4). *p < 0.05, **p < 0.01, Mann–Whitney U test.
FIGURE 4. Effect of Abs on transcriptional levels of Th17 cytokines, b-defensins, and S100As in ear skin of K5.Stat3C mice after short-term TPA treatment. Real-time RT-PCR of lesional skins revealed that anti–IL-12/23p40 (green bars) and anti–IL-23p19 (purple bars) Abs lowered transcriptional levels of Th17 cytokines, including IL-17A, IL-17F, and IL-22, b-defensin 3 (BD3), b-defensin 4 (BD4), S100A8, and S100A9, compared with the control IgG-treated group (blue bars), set to 1. Increases in Th17 cytokine mRNAs were elicited by anti–IL-17 Ab treatment (red bars) but were not statistically significant. IgG control (n = 16), anti–IL-17A (n = 8), anti–IL-12/23p40 (n = 14), anti–IL-23p19 (n = 6). *p < 0.05, **p < 0.01, Kruskal–Wallis one-way analysis.

FIGURE 5. Effect of Ab treatments on the development of bona fide TPA-induced psoriasiform lesions on the backs of K5.Stat3C mice. A, Representative macroscopic and histological views (H&E staining) of dorsal skins of mice treated with TPA following a single treatment with the indicated Abs. Scale bar, 200 μm. B, Epidermal thickness of the dorsal skin. Mean thickness ± SD (in micrometers). IgG control (n = 8), anti–IL-17A (n = 7), anti–IL-12/23p40 (n = 6), anti–IL-23p19 (n = 6). **p < 0.01, Mann–Whitney U-test.
Figure Legends for Supplemental data

FIGURE S1. Effect of antibodies on the transcriptional levels of chemokines in ear skin of K5.Stat3C mice after a short-term TPA treatment. Relative mRNA levels to Hprt transcript were shown. Pretreatment with anti-IL-17A (red bars) moderately down-regulated gene expressions of CCL1, 2, 3, and 5, compared to IgG-treated controls (blue bars), while blocking IL-12/23p40 demonstrated a greater inhibition of these transcript. IgG control (n=4), anti-IL-17A (n=4), anti-IL-12/23p40 (n=3). *, P<0.05, Mann-Whitney’s U-test.

FIGURE S2. Anti-IL-17A attenuated intraepidermal abscess formation. Representative histology of a short-term TPA treated ear skin of K5.Stat3C mice are shown. Pretreatment with control IgG (top), anti-IL-17A (bottom). Intraepidermal neutrophilic abscesses (arrows) were significantly decreased in number by anti-IL-17A. H&E. scale bar, 200µm.

FIGURE S3. Intradermal injection of IL-23 induced epidermal hyperplasia. The ear skin of K5.Stat3C mice were intradermally injected with 400ng of IL-23 for consecutive five days (bottom) and untreated (top). IL-23 induced epidermal hyperplasia without granular layer, and accumulation of neutrophils in the cornified layer, which resembled psoriasis. H&E. scale bar, 50µm.
SUPPLEMENTAL FIGURE. S1

Relative mRNA

CXCL1

CXCL2

CXCL3

CXCL5
SUPPLEMENTAL FIGURE. S2

IgG control

anti-IL-17A
SUPPLEMENTAL FIGURE. S3

untreated

IL-23 injected
## Primer sequences for genes used in the present study (mouse)

<table>
<thead>
<tr>
<th>gene</th>
<th>sense (5’-3’)</th>
<th>anti-sense (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hprt</td>
<td>CACAGGACTAGAACACCTGC</td>
<td>GCTGGTGAAAAAGGACCTCT</td>
</tr>
<tr>
<td>IL-1b</td>
<td>CAACCAACAAGTGATATTCTCATG</td>
<td>GATCCACACTCTCCAGCTGCA</td>
</tr>
<tr>
<td>IL-6</td>
<td>GCTACCAAACTGGATATAATCGGA</td>
<td>CCAGGTAGCTATGGTACTCCAGAA</td>
</tr>
<tr>
<td>IL-12p35</td>
<td>CATCAGCAGATCATCTGAGACAA</td>
<td>CGCCATTATGATTCAGAGACTG</td>
</tr>
<tr>
<td>IL-12p40</td>
<td>ATCGGTTCACGTGGTGTCCTCC</td>
<td>GGAGTCAGTCACCTCTTACA</td>
</tr>
<tr>
<td>IL-17A</td>
<td>GACTACCTCAACCGGTTCCAGTC</td>
<td>TCTACAGGGTCTTCATTGCG</td>
</tr>
<tr>
<td>IL-17F</td>
<td>CCCAGGAAGACATACTTTAAGAAA</td>
<td>CAACAGTAGCAAAAGACCTTGCA</td>
</tr>
<tr>
<td>IL-20</td>
<td>TTTTAAGGAGCAGCTGAGTCTTTTG</td>
<td>ACCCTTGCCAGATAGAATCTCA</td>
</tr>
<tr>
<td>IL-22</td>
<td>TTTCTGACCAAAACTCAGCA</td>
<td>CTGGATGGTCTGGTGTCGCA</td>
</tr>
<tr>
<td>IL-23p19</td>
<td>AGCAACCTTCAACCTCCCTAC</td>
<td>ACTGCTGACTGAAACTCAGGC</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>ACTGGCAAAAGGATGGTGAC</td>
<td>GACCTGTGGTGTGTGACCT</td>
</tr>
<tr>
<td>TGF-β</td>
<td>CACCAGCTGATATGAGAAACC</td>
<td>AAGCCCTGTATTCCGTCCT</td>
</tr>
<tr>
<td>TNF-α</td>
<td>TCTTCTCATTGCTTGGTGG</td>
<td>GGTCTGGGCCCATAAGACTGA</td>
</tr>
<tr>
<td>BD3</td>
<td>ACAATCCAGTAAAGTTGTTGAGGA</td>
<td>TCTGACGAGTGTGCCCTAGT</td>
</tr>
<tr>
<td>BD4</td>
<td>TCTCCACATTGTTCCTG</td>
<td>TGATGTTTGGATATTTGGGTAAA</td>
</tr>
<tr>
<td>S100A7</td>
<td>GCCTCGCTTTCATGACAC</td>
<td>CGGAACAGCTCTGTGATGTAGT</td>
</tr>
<tr>
<td>S100A8</td>
<td>TGCGATGGTGATATAAGTG</td>
<td>GGCCAGAAGCTCTGCAGCT</td>
</tr>
<tr>
<td>S100A9</td>
<td>CACCCCTGACCAAGGAGGAAT</td>
<td>TGTATTATTGAGGGGCTTCTT</td>
</tr>
<tr>
<td>CXCL1</td>
<td>AGACTCAGCCACACTCCAA</td>
<td>TGACAGCGCAGCTCATTG</td>
</tr>
<tr>
<td>CXCL2</td>
<td>AAAATCATCCAAAAAGATCTGAACAA</td>
<td>CTTTGGTTTTCCTGTTGGAGG</td>
</tr>
<tr>
<td>CXCL3</td>
<td>CCCAGGCTTCCAGATAATCA</td>
<td>TCTGATTTAGAATGCAGGTCTCTT</td>
</tr>
<tr>
<td>CXCL5</td>
<td>GCTGCCCTTCCCTCAGTCT</td>
<td>CACCGTAGGAGCAGCCTGGA</td>
</tr>
<tr>
<td>gene</td>
<td>sense (5'-3')</td>
<td>anti-sense (5'-3')</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>Hprt</td>
<td>TGACCTTGATTATTTTGTACTCC</td>
<td>CGAGCAAGAGCTTCAGTCTC</td>
</tr>
<tr>
<td>IL-1b</td>
<td>CTGTCTTCGCTGTTGAAGAA</td>
<td>TTGGGTAAATTTTGGGATCTCAA</td>
</tr>
<tr>
<td>Il-4</td>
<td>CACCGAGTTGACCGTACAG</td>
<td>GCCCTGCAGAAAGGTTTCC</td>
</tr>
<tr>
<td>IL-6</td>
<td>GATGAGTACAAAAAGTCCTGATCCA</td>
<td>CTGCAAGCCACTGTTTCTGT</td>
</tr>
<tr>
<td>IL-12p35</td>
<td>CACTCCAAAACCTGCTGAG</td>
<td>TCTCTCAAGATGCAAGGTTA</td>
</tr>
<tr>
<td>IL-12p40</td>
<td>CCCTGACATTCTGCATTCA</td>
<td>AGGTCTTGTCCGTAAGACTCTA</td>
</tr>
<tr>
<td>IL-17A</td>
<td>TGGGAAGACCTCATTGTTG</td>
<td>GATTTCTGAGGATGTGAT</td>
</tr>
<tr>
<td>IL-17F</td>
<td>GGCATCATCAATGAAAACCA</td>
<td>TGGGGTCCCAAGTGACAG</td>
</tr>
<tr>
<td>Il-19</td>
<td>GCCAGAGAGCACTGAGAGGA</td>
<td>AGCAGAAAGGAAACACACTG</td>
</tr>
<tr>
<td>Il-20</td>
<td>AAGAAGGAGCCTCGGGCTCT</td>
<td>TGAATCGAATCTGGCTGATTTC</td>
</tr>
<tr>
<td>Il-22</td>
<td>CAACAGGCTAAGCAATGCTCA</td>
<td>ACTGTCCTTCTCAGCTTTTTC</td>
</tr>
<tr>
<td>IL-23</td>
<td>TGTTCCTTCATCCGACTG</td>
<td>TCGCTCCAAAGCAGACTGA</td>
</tr>
<tr>
<td>IFN-g</td>
<td>CAGGTAGATTCAGAGGCTGG</td>
<td>TCTGTCAGCTCTCAGCCTTCAA</td>
</tr>
<tr>
<td>TGF-b</td>
<td>GCAGCAGTGGAAGCTGTA</td>
<td>CAGCCGGTGCTGAGGTA</td>
</tr>
<tr>
<td>TNF-a</td>
<td>CAGCCTCTTCTCCTCTCTGTC</td>
<td>GCCAGAGGCTGATTAGA</td>
</tr>
<tr>
<td>Hbegf</td>
<td>TGGGCTTCTCAGTGTTGAG</td>
<td>TGCCAAAATTCAGTCTTCTTC</td>
</tr>
<tr>
<td>Cyclind1</td>
<td>GGCAGAGAGCAGCTGAGGAGGA</td>
<td>AGCAGAAAGGAAACACACTG</td>
</tr>
<tr>
<td>Cmyc</td>
<td>AGGTGCTAAGTTGGGACAGTGCA</td>
<td>TGGTGCTTTTGGTTTGT</td>
</tr>
<tr>
<td>Involucrin</td>
<td>TTACTGTGAGTCTGTTGAG</td>
<td>TCTTGAAGGCTCAGTTGCT</td>
</tr>
<tr>
<td>Transglutaminase1</td>
<td>CCCAAGAGACAGCTAGCACTGG</td>
<td>AGACCAGGCAATCTTGTG</td>
</tr>
<tr>
<td>Keratin10</td>
<td>CCATCGATGACCTTAAAAATCAG</td>
<td>GCAGAGCCTACCTCATCCTCACTT</td>
</tr>
<tr>
<td>Keratin14</td>
<td>TTCTCCTCTGAGTGCCAGTC</td>
<td>ATGACCTTGGTGCGGATT</td>
</tr>
<tr>
<td>S100A8</td>
<td>ATGCGCGTCTACAGGAGATGC</td>
<td>AGCCCATTTTATCACAG</td>
</tr>
<tr>
<td>S100A9</td>
<td>CAGCGGAAAGCAGCAGTACA</td>
<td>TCAGCTGCTTGGTCTGCATT</td>
</tr>
<tr>
<td>BD3</td>
<td>TCCATTATCTTCTGTTTGGCTTTG</td>
<td>TCTGTAATGGTTTTATGATTCTCCAT</td>
</tr>
<tr>
<td>BD4</td>
<td>TGTCGCAAGCAAGAATACAGA</td>
<td>AGCTCTCATCCCATTGGTGTTCTCAA</td>
</tr>
</tbody>
</table>