

Inflammatory Skin Disease in *K5.hTGF- β 1* Transgenic Mice Is Not Dependent on the IL-23/Th17 Inflammatory Pathway

Erin L. Fitch^{1,2}, Heather L. Rizzo¹, Stephen E. Kurtz³, Keith W. Wegmann⁴, Wei Gao⁵, Jacqueline M. Benson⁵, David J. Hinrichs⁴ and Andrew Blauvelt^{1,2,3}

In the presence of IL-6, transforming growth factor (TGF)- β 1 induces differentiation of T helper (Th) 17 cells in mice. Interleukin (IL)-23, a heterodimeric cytokine composed of IL-23p19 and IL-12/23p40 subunits, stimulates the growth and expansion of Th17 cells, and has been implicated in psoriasis pathogenesis. To study the associations between TGF- β 1, the IL-23/Th17 inflammatory pathway, and psoriasis, we investigated inflammatory skin disease in transgenic mice that constitutively overexpress human TGF- β 1 in basal keratinocytes (*K5.hTGF- β 1* transgenic mice); these mice had previously been reported as having a psoriasis-like disease. *K5.hTGF- β 1* transgenic mice had high levels of TGF- β 1 mRNA and protein in both skin and serum. Levels of cytokines involved in IL-23/Th17-mediated inflammation were not elevated in lesional skin compared with those in non-lesional and wild-type skin. It is noteworthy that IL-4 and IgE were markedly elevated in inflamed skin and serum, respectively, of transgenic mice. Monoclonal antibodies (mAbs) specifically directed against IL-23p19 or IL-12/23p40 had no clinical effect on established inflammatory skin disease in *K5.hTGF- β 1* transgenic mice, whereas the same mAbs were able to block the development of murine experimental autoimmune encephalomyelitis, an IL-23/Th17-mediated disease. In summary, the IL-23/Th17 inflammatory pathway is not responsible for the maintenance of inflammatory skin disease in *K5.hTGF- β 1* transgenic mice.

Journal of Investigative Dermatology advance online publication, 9 April 2009; doi:10.1038/jid.2009.88

INTRODUCTION

Interleukin (IL)-23 is primarily produced by dendritic cells (Oppmann *et al.*, 2000), and is clearly elevated in psoriasis lesions, as indicated by increased levels of mRNA for each of its subunit components, *IL-23p19* and *IL-12/23p40*, in lesional skin as compared with those in non-lesional skin, whereas mRNA levels of *IL-12/35p35* are not elevated (Lee *et al.*, 2004; Chamian *et al.*, 2005; Chan *et al.*, 2006; Toichi *et al.*, 2006). Furthermore, an intradermal injection of recombinant IL-23, but not of IL-12, into mice induces acute histological changes reminiscent of human psoriasis (Chan

et al., 2006; Zheng *et al.*, 2007). These IL-23-triggered changes are dependent on cytokines produced by T helper (Th) 17 cells (Zheng *et al.*, 2007). Th17 cells are a newly appreciated subset of CD4+ T helper cells, distinct from Th1 and Th2 cells, which are dependent on IL-23 for survival and expansion (Oppmann *et al.*, 2000; Aggarwal *et al.*, 2003). Genetic or antibody targeting of either IL-23 or Th17 cells ameliorates several T-cell-mediated autoimmune disorders in mice, including classic models of rheumatoid arthritis and multiple sclerosis (Cua *et al.*, 2003; Murphy *et al.*, 2003; Langrish *et al.*, 2005; Chen *et al.*, 2006). In contrast, targeting of IL-12 or Th1 cells leads to worsening of these same conditions (Cua *et al.*, 2003; Murphy *et al.*, 2003; Langrish *et al.*, 2005; Chen *et al.*, 2006). Thus, the IL-23/Th17 inflammatory pathway is critical in mediating distinct autoimmune T-cell-mediated inflammatory diseases in mice, and its role in mediating inflammation in human psoriasis appears to be equally important (Fitch *et al.*, 2007).

Although IL-23 stimulates Th17 survival and proliferation, differentiation of murine Th17 cells from naive T-cell precursors is dependent on transforming growth factor (TGF)- β 1 in combination with IL-6 (Bettelli *et al.*, 2006; Mangan *et al.*, 2006; Veldhoen *et al.*, 2006). Neutralization of TGF- β 1 abrogates differentiation of naive murine CD4+ T cells to Th17 cells in culture, whereas exposure to TGF- β 1 and pro-inflammatory cytokines, such as IL-6 induces Th0

¹Department of Dermatology, Oregon Health & Science University, Portland, Oregon, USA; ²Department of Molecular Microbiology and Immunology, Oregon Health & Science University, Portland, Oregon, USA; ³Dermatology Service, Veterans Affairs Medical Center, Portland, Oregon, USA; ⁴Department of Immunology, Veterans Affairs Medical Center, Portland, Oregon, USA and ⁵Centocor Research & Development, Radnor, Pennsylvania, USA

Correspondence: Dr Andrew Blauvelt, Department of Dermatology, Oregon Health & Science University, 3710 SW US Veterans Hospital Drive, Mail Code R&D 55, Portland, Oregon 97239, USA. E-mail: blauvean@ohsu.edu

Abbreviations: CCR, chemokine receptor; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; mAb, monoclonal antibody; qRT-PCR, quantitative real-time reverse transcriptase polymerase chain reaction; TGF, transforming growth factor; Th, T helper; Treg, regulatory T cell; WT, wild-type

Received 8 May 2008; revised 12 February 2009; accepted 11 March 2009

cells to become mature Th17 effector cells (Veldhoen *et al.*, 2006). Mice that completely lack TGF- β 1 have lower serum levels of IL-17 and produce fewer IL-17+CD4+ splenic T cells, whereas mice that overexpress TGF- β 1 under the IL-2 promoter produce more antigen-stimulated IL-17 (Mangan *et al.*, 2006). In addition, TGF- β 1 expression by T cells is required for the induction of Th17 cells in certain tissues (Li *et al.*, 2007).

K5.hTGF- β 1 transgenic mice overexpress latent human TGF- β 1 in basal keratinocytes and hair follicles under the *K5* promoter, which leads to the development of inflammatory skin disease (Li *et al.*, 2004). A recent evaluation of murine models of human psoriasis ranked *K5.hTGF- β 1* transgenic mice high among the numerous models, because of clinical, histological, and cellular similarities to human psoriasis (Gudjonsson *et al.*, 2007). These mice clinically showed many clinical and cellular hallmarks of psoriasis, including erythematous scaly plaques, epidermal hyperproliferation, hyperkeratosis, acanthosis, and inflammatory cell infiltrates of CD8+ T cells and neutrophils in the epidermis, and CD4+ T cells and macrophages in the dermis (Li *et al.*, 2004). Clinical disease is apparent by 4 months of age in most mice, with consistent Koebnerization of ears at sites of ear tagging (Li *et al.*, 2004). As TGF- β 1 induces Th17 differentiation in mice, and given the importance of Th17 cells in human psoriasis, we investigated the role of the IL-23/Th17 inflammatory pathway in the pathogenesis of inflammatory skin disease in *K5.hTGF- β 1* transgenic mice.

RESULTS

mRNA levels of IL-23/Th17 pathway cytokines are not elevated in the lesional skin of *K5.hTGF- β 1* transgenic mice

To test the hypothesis that inflammatory skin disease in *K5.hTGF- β 1* transgenic mice is because of the increased presence of Th17 cells, we measured mRNA levels of several cytokines that are important in the IL-23/Th17 inflammatory pathway, using quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) (Figure 1). Data are expressed in three separate ways: comparison of lesional skin with wild-type (WT) skin (Figure 1a); comparison of clinically normal-appearing (that is, non-lesional) skin with WT skin (Figure 1b); and comparison of lesional skin with non-lesional skin (Figure 1c). As expected, lesional and non-lesional skin contained high levels of *hTGF- β 1* mRNA. *TNF- α* , *IL-6*, and *IL-17A* mRNAs were also elevated in lesional skin. However, mRNAs specific for the *IL-23p19* and *IL-12/23p40* subunits of IL-23, as well as for *IL-23R*, a component of the IL-23 receptor expressed on Th17 cells, were present at similar levels in both lesional and non-lesional skin as those in WT skin. mRNA encoding the Th17 cell-associated transcription factor, *ROR γ t*, was present at decreased levels compared with that in WT skin. mRNA encoding IL-22, a key Th17 effector cytokine, was not detected in any skin sample.

We next examined cytokines associated with Th1, Th2, and regulatory T-cell (Treg) responses using qRT-PCR (Figure 1). *IL-12/35p35* levels were similar in both lesional and WT skins. It is interesting that levels of *IL-4*, a Th2

cytokine, were high in transgenic lesional skin compared with those in WT skin. Compared with non-lesional skin, lesional skin had elevated mRNA levels for Treg-associated cytokines, including *foxp3*, *IL-10*, and *IL-12p35*, which is a component of IL-12 and of a newly described and crucial Treg cytokine, IL-35 (Collison *et al.*, 2007).

IL-23/Th17 cytokine protein levels are not detectable in lesional skin of *K5.hTGF- β 1* transgenic mice

We isolated total protein from lesional and non-lesional skin of *K5.hTGF- β 1* transgenic mice and analyzed tissue and serum samples for the presence of IL-23 and Th17-related cytokines by enzyme-linked immunosorbent assay (ELISA) (Figure 2). Transgenic mice exhibited high levels of total hTGF- β 1 protein (Figure 2a). IL-1 α protein was detected at similar levels in skin obtained from both *K5.hTGF- β 1* transgenic mice and WT mice (data not shown). Protein levels of IFN- γ were slightly elevated and IL-4 levels were markedly elevated in lesional skin (Figure 2b and c). As a result of the latter finding, we tested the serum of transgenic mice, and found serum IgE to be very high in *K5.hTGF- β 1* transgenic mice compared with that in WT mice (Figure 2d). In contrast, IL-17A, IL-22, and IL-23 proteins were not detected in skin of *K5.hTGF- β 1* transgenic mice or in that of WT mice. WT and transgenic lymph node cultures treated with IL-17-promoting cytokines showed high levels of IL-17A, IL-22, and IL-12/23p40. IL-12/23p40 was also detected in sera of both groups of mice. No differences were observed in IL-17A, IL-22, and IL-12/23p40 protein levels in the lymph node and sera between WT and *K5.hTGF- β 1* transgenic mice (Figure 2e and h).

Treatment with neutralizing antibodies directed against IL-23 subunits, IL-23p19 or IL-12/23p40, does not improve established inflammatory skin disease in *K5.hTGF- β 1* transgenic mice

To determine the functional role of the IL-23/Th17 axis in mice with advanced skin disease, we neutralized specific components of the pathway with monoclonal antibodies (mAbs), as has been done recently in humans with psoriasis (Krueger *et al.*, 2007; Kimball *et al.*, 2008; Leonardi *et al.*, 2008; Papp *et al.*, 2008). Treated mice were evaluated with a clinical disease-scoring system to determine the response to therapy. Mice with baseline disease scores of two or more were treated for 3 weeks by intraperitoneal injection, with 1 mg of neutralizing mAbs directed against either IL-23p19 or IL-12/23p40, or they were treated with isotype control Abs. Skin disease progressed in a similar manner in isotype control Ab-treated (Figures 3a and 4a), anti-IL-23p19 mAb-treated (Figures 3b and 4a), and anti-IL-12/23p40 mAb-treated (Figures 3c and 4a) *K5.hTGF- β 1* transgenic mice. It is noteworthy that all histological analyses of lesional skin isolated from *K5.hTGF- β 1* transgenic mice undergoing treatment were similar, and showed hyperkeratosis with serum crust, acanthosis, and a superficial mixed inflammatory infiltrate consisting of mononuclear cells and scattered eosinophils (Figure 3). Weight loss was similar across all groups tested (Figure 4b). To ensure that mAbs were

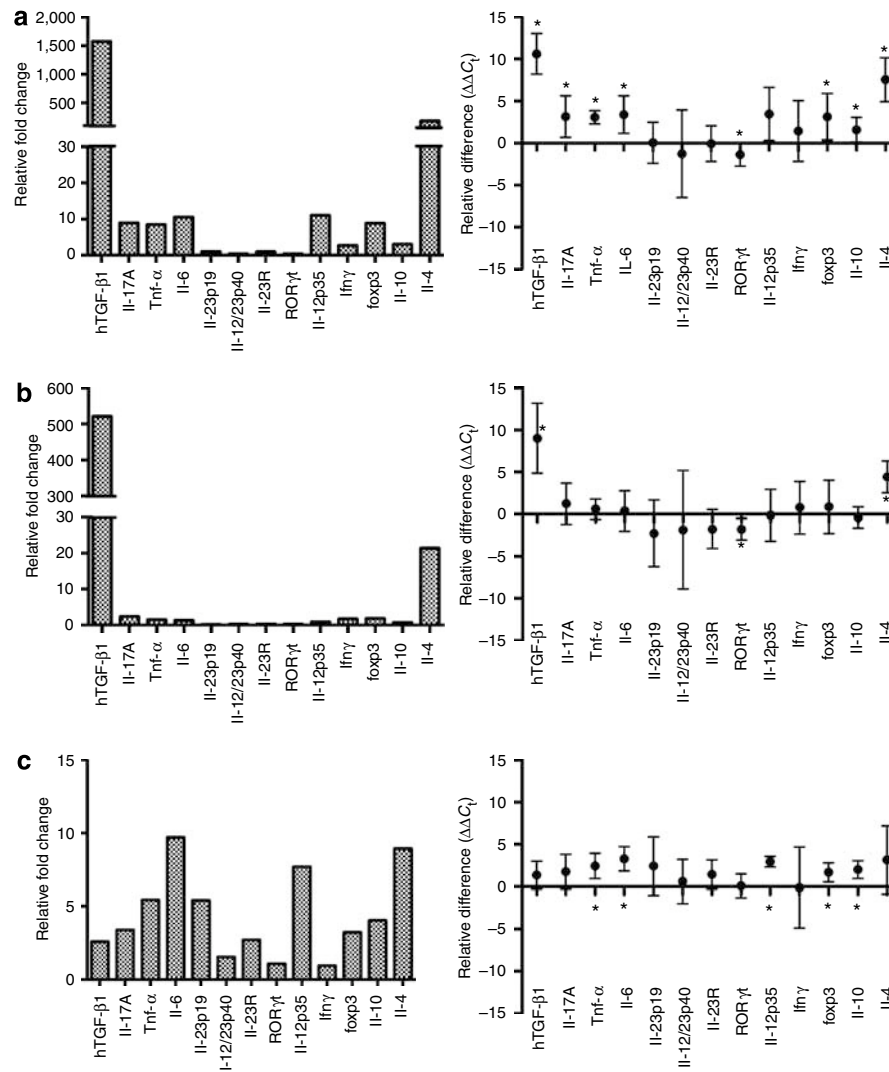


Figure 1. mRNA levels of IL-23/Th17 pathway cytokines are not elevated in lesional skin of *K5.hTGF- β 1* transgenic mice. Total RNA was isolated from the skin of WT mice ($n=4$), and from the lesional ($n=7$) and non-lesional ($n=4$) skin of *K5.hTGF- β 1* transgenic mice, and cDNA was synthesized as detailed in the Materials and Methods section. Data are presented as fold change (left column) or relative difference with 95% confidence interval for (a) lesional skin compared with WT skin, (b) non-lesional skin compared with WT skin, and (c) lesional skin compared with non-lesional skin. * $P<0.05$ using a two-tailed t -test, with four to nine degrees of freedom and 95% confidence intervals for graphing.

functional in neutralizing IL-12 and IL-23, treatment with 1 mg of anti-IL-23p19 and anti-IL-12/23p40 mAbs prevented the onset of experimental autoimmune encephalomyelitis (Figure 4c), a disease mediated by IL-23 and Th17 cells (Chen *et al.*, 2006).

DISCUSSION

The results presented here indicate that the IL-23/Th17 inflammatory pathway is not responsible for maintenance of inflammatory skin disease in *K5.hTGF- β 1* transgenic mice. As TGF- β 1 is a critical component in the development of Th17 cells in mice (Bettelli *et al.*, 2006; Mangan *et al.*, 2006; Veldhoen *et al.*, 2006), we originally hypothesized that overexpression of TGF- β 1 by keratinocytes could influence the differentiation of naive T cells in skin-draining lymph

nodes, and lead to enhanced development of Th17 cells. These Th17 cells, through expression of skin-homing receptors, CCR6 and CCR4 (Acosta-Rodriguez *et al.*, 2007a, b), would return to the skin and release inflammatory cytokines, such as IL-17A, IL-22, and TNF- α . The net result would be the establishment and maintenance of psoriasisform lesions (Li *et al.*, 2004). Disease would resolve on mAb therapy directed against IL-23, similar to human psoriasis (Krueger *et al.*, 2007; Kimball *et al.*, 2008; Leonardi *et al.*, 2008; Papp *et al.*, 2008). We showed, however, that levels of IL-23 and Th17 cytokines are not elevated in *K5.hTGF- β 1* transgenic mice (Figures 1 and 2). We also showed that mAb targeting of the IL-23/Th17 inflammatory pathway does not affect the maintenance of skin disease in these mice (Figures 3 and 4). As we learn more about the role of IL-23

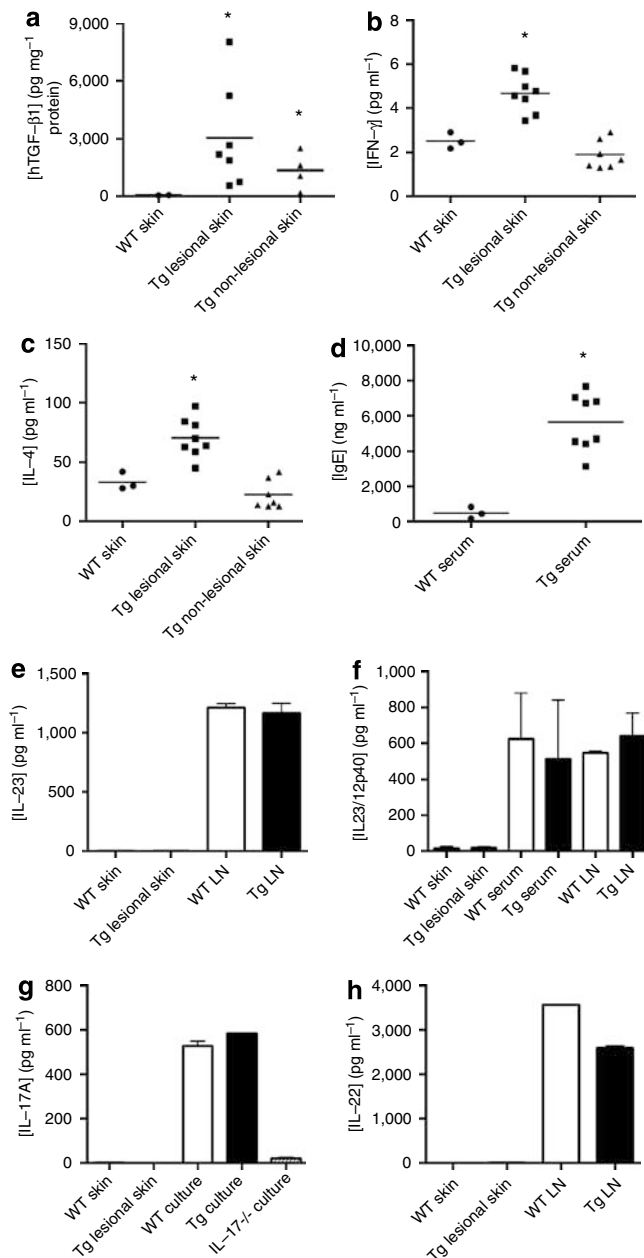


Figure 2. IL-23/Th17 cytokine protein levels are not detectable in lesional skin of *K5.hTGF- β 1* transgenic mice. Total protein was isolated from the skin of WT mice ($n=3$), and from the lesional ($n=4-8$) and non-lesional ($n=7-8$) skin of *K5.hTGF- β 1* transgenic mice. Protein was assayed by ELISA. (a) human TGF- β 1; (b) IFN- γ ; (c) IL-4; (d) serum IgE; (e) IL-23p19/p40; (f) IL-12/23p40; (g) IL-17A; and (h) IL-22. (a-c and e-h) * $P<0.05$ comparing WT with transgenic samples using a one-way analysis of variance with Tukey's post test. (d) * $P<0.0005$ comparing WT with transgenic serum using an unpaired t -test.

and Th17 cells in the pathogenesis of human psoriasis and other inflammatory skin diseases, it will be critical to investigate this pathway in each of the various murine models of these diseases. We believe that this study will provide the framework to carry out these future studies.

There are some limitations to our study. The *K5.hTGF- β 1* transgenic mice showed clinical, histological, and cytokine

expression features that were different than those originally described by Li *et al.* (2004). More specifically, this original publication described many characteristics of human psoriasis, yet our findings suggest that these mice have features that resemble human atopic dermatitis more so than human psoriasis (for example, crusted skin lesions, scattered tissue eosinophils, high lesional IL-4 expression, and high-serum IgE levels). It is unclear why we found such different results compared with the results arrived at by Li *et al.*, but this may be because of changes in transgene expression that could occur after years of breeding of *K5.hTGF- β 1* transgenic mice. Although we have definitively shown that the IL-23/Th17 inflammatory pathway is not involved in the inflammatory skin disease observed in these transgenic mice, the precise mechanisms underlying pathogenesis in these mice remain unresolved. For example, the contributions of bacterial colonization and scratching in exacerbating skin lesions in these mice may be studied further, and would be particularly relevant in further understanding the pathogenesis of atopic dermatitis.

TGF- β 1 expression can paradoxically activate and suppress the immune function (Wan and Flavell, 2007). Although the combination of TGF- β 1 and IL-6 can direct the differentiation of murine Th17 cells, TGF- β 1 can also inhibit the pathogenic function of these cells, even in the presence of IL-23 (McGeachy *et al.*, 2007). It is possible that TGF- β 1, in the setting of *K5.hTGF- β 1* transgenic mice, does not drive Th17 cells toward an inflammatory phenotype, but restricts their pro-inflammatory activities. Furthermore, TGF- β 1, in a non-inflammatory tissue milieu, can induce Treg differentiation from naive T cells as well as the production of the Treg transcription factor, *foxp3* (McGeachy and Cua, 2008). Our data indicate that mRNA levels of Treg-associated factors, *foxp3*, *IL-12/35p35*, and *IL-10*, are elevated in lesional skin than in non-lesional skin. It is also noteworthy that the protein subunit encoded by *IL-12/35p35* can bind with the Epstein-Barr virus-induced 3 protein, or EB13, to create the anti-inflammatory Treg-associated cytokine known as IL-35 (Collison *et al.*, 2007). Although *foxp3*, *IL-12/35p35*, and *IL-10* mRNA are elevated in skin of *K5.hTGF- β 1* transgenic mice, it is still not known whether Treg populations play a key pathogenic role in these mice.

TGF- β 1 has an unclear role in the pathogenesis of human psoriasis. In one study of 60 patients with psoriasis and 38 without psoriasis, serum TGF- β 1 was increased in psoriatics than in controls, and higher TGF- β 1 correlated with higher clinical disease scores (Nockowski *et al.*, 2004). Another study showed a correlation of disease severity with concentrations of TGF- β 1 in psoriatic scales (Flisiak *et al.*, 2002). Several other studies, however, have shown a negative or absent correlation of expression of TGF- β 1 or its receptors in psoriasis (Leivo *et al.*, 1998; Doi *et al.*, 2003). These published studies are insufficient to determine definitely whether there is a positive or negative correlation between TGF- β 1 and psoriasis. Indeed, recent studies showed that TGF- β 1 is not involved in the differentiation of human Th17 cells (unlike in the development of murine Th17 cells)

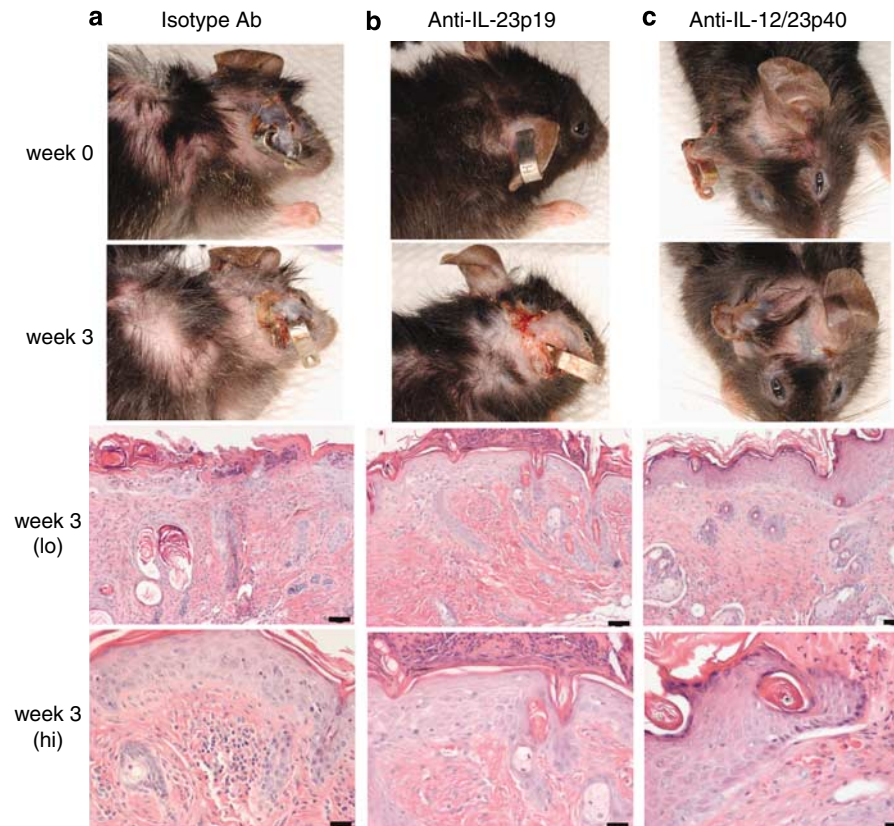


Figure 3. Treatment with neutralizing antibodies directed against IL-23 subunits, IL-23p19, or IL-12/23p40, does not improve established inflammatory skin disease in *K5.hTGF- β 1* transgenic mice. *K5.hTGF- β 1* transgenic mice were treated on weeks 0, 1, and 2 with 1 mg isotype control Ab (a), anti-IL-23p19 mAbs (b), or anti-IL-12/23p40 mAbs (c). $n=5$ mice per group for anti-IL-23p19 and anti-IL-12/23p40, and $n=4$ mice per group for isotype. (a–c) Representative clinical photos are shown before (week 0) and after treatment (week 3), whereas representative H&E skin sections are shown after treatment at week 3. It is interesting to note that all histological analyses of lesional skin isolated from *K5.hTGF- β 1* transgenic mice undergoing treatment were similar, and showed hyperkeratosis with serum crust, acanthosis, and a superficial mixed inflammatory infiltrate consisting of mononuclear cells and scattered eosinophils. Bar = 40 μ m in figures labeled “lo” and 20 μ m in figures labeled “hi.”

(Acosta-Rodriguez *et al.*, 2007a,b), and that there is no association between TGF- β 1 polymorphisms and psoriasis susceptibility (Baran *et al.*, 2007). Taken together, our current study, as well as these recent studies in humans, suggests that TGF- β 1 does not play a major role in the pathogenesis of psoriasis.

MATERIALS AND METHODS

Animals

All breeding and experiments were undertaken with review and approval from the Portland Veterans Affairs Medical Center Institutional Animal Care and Use Committee. *K5.TGF- β 1* transgenic mice were a kind gift from Dr Xiao-Jing Wang, University of Colorado (Li *et al.*, 2004). C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). To ensure the proper genetic identification of mice, DNA was isolated from tails of mice at 3 weeks of age using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. PuReTaq Ready-To-Go PCR beads (GE Healthcare, Piscataway, NJ) were used for DNA amplification, following the manufacturer's instructions. Primer sequences and product sizes were as follows: *K5.hTGF- β 1* forward, 5'-GCGTCTGCTGAGGCTCAAGTT-3' and

reverse 5'-ACCTCGGCGGCCGCTAG-3', 271 bp. The annealing temperature for *K5.hTGF β 1* primers was 54°C.

Scoring of inflammatory skin disease

All mice were monitored from birth to determine the extent, if any, of skin or systemic disease. *K5.hTGF- β 1* transgenic mice developed a visible skin disease between 2 and 4 months of age, with disease being somewhat variable among individual animals. A skin-scoring system was instituted to standardize all experiments involving cytokine levels, treatment of established disease, and genetic crosses. Scores were assessed as follows: 0.5 = altered hair growth; 1 = thinning of ventral and dorsal hair and mild scaling on the upper back; 2 = <50% areas with hair loss, widespread scaling, and Koebner's phenomenon near ear tags; 3 $\geq$ 50% areas with hair loss and erythematous scaly plaques; and 4 $\geq$ 50% areas with hair loss, erythematous scaly plaques, retracted eyelids, and dehydration. All animals that were used for measurement of cytokine levels were between 4 and 6 months of age. The mice were euthanized at the end of the experiments by CO₂, on >10% weight loss, or if showing obvious dehydration. The mice were housed in specific pathogen-free facilities and given food and water *ad libitum*.

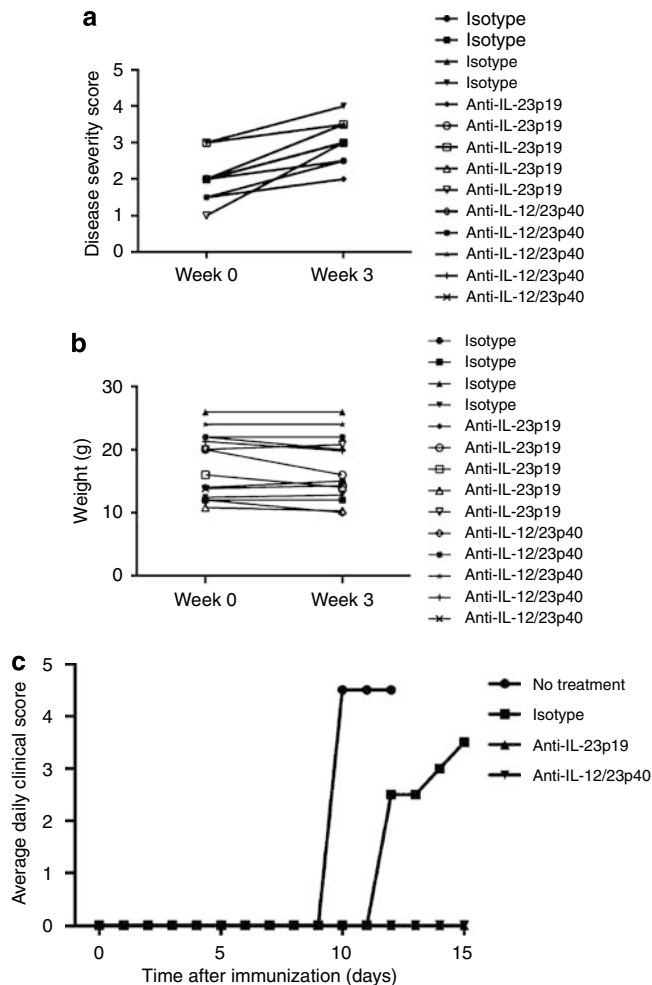


Figure 4. Skin disease score and weight do not improve after treatment of *K5.hTGF- β 1* transgenic mice with neutralizing antibodies directed against IL-23 subunits, IL-23p19, or IL-12/23p40. (a) Severity of disease was assessed at weeks 0 and 3. (b) Weights of mice were assessed at weeks 0 and 3, as an additional quantitative measurement of disease. (c) Anti-IL-23p19 and anti-IL-12/23p40 mAbs were effective in preventing experimental autoimmune encephalomyelitis in SJL mice. Mice were injected i.p. on days -1 and 6 with 1 mg of anti-IL-23p19 or anti-IL-12/23p40 mAbs, or isotype IgG Abs, or nothing. Disease severity was measured daily after immunization with PLP139-151.

RNA isolation and qRT-PCR

Clinically abnormal (that is, lesional) and clinically normal (that is, non-lesional) skins were obtained from C57BL/6 and *K5.hTGF- β 1* transgenic mice, snap frozen in liquid nitrogen, placed into Trizol (Sigma, St Louis, MO), and homogenized with a mechanical rotor for 30 seconds. RNA was isolated according to standard Trizol protocol, and was followed by further purification using an RNeasy kit (Qiagen). cDNA was prepared from 1 μ g of total RNA by reverse transcription using iScript (Bio-Rad, Hercules, CA). Five reactions were pooled and diluted 5 times with water to establish the same pool of cDNA for all qRT-PCR experiments. qRT-PCR was performed with TaqMan primers and fluorescent probes for *GAPDH*, *hTGF- β 1*, *IL-23p19*, *IL-12/23p40*, *IL-12/35p35*, *IL-23R*, *IL-6*, *IL-17A*, *IL-22*, *IFN- γ* , *ROR γ t*, *CCR6*, *IL-4*, *foxp3*, and *IL-10* (Applied Biosystems, Carlsbad, CA) on the MyiQ system (Bio-Rad). Relative quantification

by the delta-delta C_t method was carried out in Excel, using *GAPDH* as the housekeeping gene. To generate logarithmic graphs, 100 was added to all observations and the log of each was calculated. WT results were fixed at two (log 100). Gains relative to WT were shown as greater than two and losses were shown as less than two.

Protein isolation and ELISA

Sera, lesional skin, and non-lesional skin were obtained from C57BL/6 and *K5.hTGF- β 1* transgenic mice. Skin was snap frozen in liquid nitrogen, pulverized when immersed in liquid nitrogen, and subsequently re-suspended in a protein lysis buffer (10 mM Tris-HCl, pH 7.5; 0.5 mM EDTA- Na_2 ; 0.5 mM EGTA; 1% Triton X-100; 0.5 mM PMSF; and Protease Inhibitor Mix, diluted from 100 $\times$ stock (Sigma)). The samples underwent three freeze-thaw cycles and were spun at full speed for 15 minutes to fractionate the sample and remove protein. Human TGF- β 1 and murine IL-17A, IL-22, IL-1 α , TGF- β 1, and IL-12/23p40 ELISA kits (R&D Systems, Minneapolis, MN), the IL-23 p19/p40, IFN- γ , and IL-4 ELISA kits (eBiosciences, San Diego, CA), and the IgE ELISA kit (Biolegend, San Diego, CA) were used according to the manufacturer's instructions.

Cell culture

Lymph nodes from WT, *K5.hTGF- β 1* transgenic, or *IL-17A* knockout mice were harvested and cultured for 3 days in RPMI-1640, 5% FBS, 2 mM L-glutamine, and 100 U ml $^{-1}$ penicillin/100 μ g ml $^{-1}$ streptomycin. Cells were cultured alone or in the presence of IL-17-promoting cytokines or antibodies, including hTGF- β 1, IL-6, anti-IFN- γ , and recombinant murine IL-23 (Li *et al.*, 2007). The cells were plated at a density of 5×10^6 cells per ml in six-well plates. Then, 150 μ l aliquots of cell culture supernatant, to use as protein assay controls, were taken at 24-hour intervals, beginning at 12 hours. The culture medium was supplemented with 1 ml new medium on day 3.

Treating established inflammatory skin disease in *K5.hTGF- β 1* transgenic mice

Neutralizing mAbs directed against murine IL-23p19 (CNTO 6163) and IL-12/23p40 (CNTO 3913), as well as murine IgG isotype control antibody (CNTO 1322), were obtained from Centocor R&D (Radnor, PA). Generation and *in vitro* and *in vivo* characterization CNTO 3913 and CNTO 1322 have been described elsewhere (Held *et al.*, 2008). CNTO 6163 is a rat (v)/mouse(c) IgG $_{2a}$ chimeric antibody developed by Centocor using variable regions from the neutralizing rat anti-mouse IL-23p19 antibody, CNTO 209. Functional variable region genes were identified from the hybridoma cell line and the heavy-chain and light-chain variable-region genes were cloned into murine IgG $_{2a}$ and Kappa expression vectors, respectively. Both single-gene vectors were combined into a single double-gene vector, which was transfected into GS-CHO cells to generate a stable cell line expressing the CNTO 6163 antibody.

A concentration of 1 mg of mAb per animal was used. The animals were paired by disease score and birth month and placed into the anti-IL-23p19, anti-IL-12/IL-23p40, or isotype control Ab treatment groups. Intraperitoneal (i.p.) injections were given once weekly for 3 weeks. Weight changes were monitored weekly, and clinical skin disease was scored between days 0 and 21. The animals were killed on day 21, and lesional and non-lesional skin samples were prepared for routine histology.

Treating experimental autoimmune encephalomyelitis

Female SJL mice (8–12 weeks old) were immunized subcutaneously with PLP139–151 peptide (Genscript, Piscataway, NJ) emulsified in CFA, supplemented with 2 mg ml⁻¹ *Mycobacterium tuberculosis* (Difco, San Jose, CA). The mice ($n=3$ per group) were injected i.p. with 1 mg anti-IL-23p19 mAb, anti-IL-12/23p40 mAb, or isotype control IgG at days -1 and 6. Clinical disease was assessed daily using a scoring system as follows: 0=normal; 0.5=limp tail; 1=hind limb weakness; 1.5=inability to right>; 2=walks with difficulty; 2.5=hind limb paresis; 3=complete hind limb paralysis; 3.5=hind limb paralysis with front limb weakness; 4=hind limb paralysis with front limb paresis; and 5=moribund/death. Disease was scored daily until day 15.

Statistical analyses

Statistical analyses for qRT-PCR results were carried out using a two-tailed *t*-test, with four to nine degrees of freedom and 95% confidence intervals for graphing. Statistical analyses for protein results comparing WT with transgenic samples were carried out using a one-way analysis of variance with Tukey's post test, except for the serum IgE results, which were compared using an unpaired *t*-test.

CONFLICT OF INTEREST

Dr A Blauvelt is a scientific consultant and clinical investigator for Centocor and Abbott. Jacqueline Benson and Wei Gao are employees of Centocor.

ACKNOWLEDGMENTS

This work was supported by a Veterans Affairs Merit Award (A.B.) and US National Institutes of Health Grant nos. R21 AR054495-01A1 (A.B.) and T32 CA106195-04 (E.L.F.). We greatly appreciate receiving the *K5.hTGF- β 1* transgenic mice from Dr Xiao-Jing Wang at the University of Colorado. We also thank Michael Lasarev for help with statistical analysis of the RT-PCR data and Jill M. Carton for helping produce the Centocor monoclonal antibodies used in this study.

REFERENCES

- Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F (2007a) Interleukins 1 β and 6 but not transforming growth factor- β are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* 8:942–9
- Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A *et al.* (2007b) Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat Immunol* 8:639–46
- Aggarwal S, Ghilardi N, Xie MH, de Sauvage FJ, Gurney 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
- Baran W, Szepietowski JC, Mazur G, Baran E (2007) TGF- β (1) gene polymorphism in psoriasis vulgaris. *Cytokine* 38:8–11
- Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M *et al.* (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441:235–8
- Chamian F, Lowes MA, Lin SL, Lee E, Kikuchi T, Gilleaudeau P *et al.* (2005) Alefacept reduces infiltrating T cells, activated dendritic cells, and inflammatory genes in psoriasis vulgaris. *Proc Natl Acad Sci USA* 102:2075–80
- Chan JR, Blumenschein W, Murphy E, Diveu C, Wiekowski M, Abbondanzo S *et al.* (2006) IL-23 stimulates epidermal hyperplasia via TNF and IL-20R2-dependent mechanisms with implications for psoriasis pathogenesis. *J Exp Med* 203:2577–87
- Chen Y, Langrish CL, McKenzie B, Joyce-Shaikh B, Stumhofer JS, McClanahan T *et al.* (2006) Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. *J Clin Invest* 116:1317–26
- Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM *et al.* (2007) The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 450:566–9
- Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B *et al.* (2003) Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 421:744–8
- Doi H, Shibata MA, Kiyokane K, Otsuki Y (2003) Downregulation of TGF β isoforms and their receptors contributes to keratinocyte hyperproliferation in psoriasis vulgaris. *J Dermatol Sci* 33:7–16
- Fitch E, Harper E, Skorchewa I, Kurtz SE, Blauvelt A (2007) Pathophysiology of psoriasis: recent advances on IL-23 and Th17 cytokines. *Curr Rheumatol Rep* 9:461–7
- Flisiak I, Chodnicka B, Porebski P, Flisiak R (2002) Association between psoriasis severity and transforming growth factor β (1) and β (2) in plasma and scales from psoriatic lesions. *Cytokine* 19:121–5
- Gudjonsson JE, Johnston A, Dyson M, Valdimarsson H, Elder JT (2007) Mouse models of psoriasis. *J Invest Dermatol* 127:1292–308
- Held KS, Glass WG, Orlovsky YI, Shamberger KA, Petley TD, Branigan PJ *et al.* (2008) Generation of a protective T cell response following coronavirus infection of the central nervous system is not dependent on IL-12/23 signaling. *Viral Immunol* 21:173–88
- Kimball AB, Gordon KB, Langley RG, Menter A, Chartash EK, Valdes J (2008) Safety and efficacy of ABT-874, a fully human interleukin 12/23 monoclonal antibody, in the treatment of moderate to severe chronic plaque psoriasis: results of a randomized, placebo-controlled, phase 2 trial. *Arch Dermatol* 144:200–7
- Krueger GG, Langley RG, Leonardi C, Yeilding N, Guzzo C, Wang Y *et al.* (2007) A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. *N Engl J Med* 356:580–92
- Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD *et al.* (2005) IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 201:233–40
- Lee E, Trepicchio WL, Oestreicher JL, Pittman D, Wang F, Chamian F *et al.* (2004) Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. *J Exp Med* 199:125–30
- Leivo T, Leivo I, Kariniemi AL, Keski-Oja J, Virtanen I (1998) Down-regulation of transforming growth factor- β receptors I and II is seen in lesional but not non-lesional psoriatic epidermis. *Br J Dermatol* 138:57–62
- Leonardi CL, Kimball AB, Papp KA, Yeilding N, Guzzo C, Wang Y *et al.* (2008) Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomized, double-blind, placebo-controlled trial (PHOENIX 1). *Lancet* 371:1665–74
- Li AG, Wang D, Feng XH, Wang XJ (2004) Latent TGF β 1 overexpression in keratinocytes results in a severe psoriasis-like skin disorder. *EMBO J* 23:1770–81
- Li MO, Wan YY, Flavell RA (2007) T cell-produced transforming growth factor- β 1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. *Immunity* 26:579–91
- Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO *et al.* (2006) Transforming growth factor- β induces development of the T(H)17 lineage. *Nature* 441:231–4
- McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T *et al.* (2007) TGF- β and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat Immunol* 8:1390–7
- McGeachy MJ, Cua DJ (2008) Th17 cell differentiation: the long and winding road. *Immunity* 28:445–53
- Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA *et al.* (2003) Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. *J Exp Med* 198:1951–7

- Nockowski P, Szepietowski JC, Ziarkiewicz M, Baran E (2004) Serum concentrations of transforming growth factor β 1 in patients with psoriasis vulgaris. *Acta Dermatovenerol Croat* 12:2-6
- Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B *et al.* (2000) Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 13:715-25
- Papp KA, Langley RG, Lebwohl M, Krueger GG, Szapary P, Yeilding N *et al.* (2008) Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2). *Lancet* 371:1675-84
- Toichi E, Torres G, McCormick TS, Chang T, Mascelli MA, Kauffman CL *et al.* (2006) An anti-IL-12p40 antibody down-regulates type 1 cytokines, chemokines, and IL-12/IL-23 in psoriasis. *J Immunol* 177:4917-26
- Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B (2006) TGF β in the context of an inflammatory cytokine milieu supports *de novo* differentiation of IL-17-producing T cells. *Immunity* 24:179-89
- Wan YY, Flavell RA (2007) 'Yin-Yang' functions of transforming growth factor- β and T regulatory cells in immune regulation. *Immunol Rev* 220:199-213
- Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J *et al.* (2007) Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* 445:648-51