A Role for TGFβ Signaling in the Pathogenesis of Psoriasis

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Deregulation of transforming growth factor-β (TGFβ) signaling has been reported in human psoriasis. Our recent study using a keratin 5 promoter (K5.TGFβ1wt) showed that transgenic mice expressing wild-type TGFβ1 in the epidermis developed severe skin inflammation. Additional experimental data further support a direct role for TGFβ1 overexpression in skin inflammation. First, we temporally induced TGFβ1 expression in keratinocytes in our gene-switch TGFβ1wt transgenic mice and found inflammation severity correlated with TGFβ1wt transgene expression. Second, deletion of T cells in K5.TGFβ1wt mice significantly delayed skin inflammation and associated epidermal hyperplasia/hyperkeratinosis. Third, therapeutic approaches effective for human psoriasis, that is, Etanercept and Rosiglitazone, are effective in alleviating the symptoms observed in K5.TGFβ1wt mice. Future studies will analyze specific mechanisms and identify key factors in TGFβ1-induced skin inflammation. Our mouse models will provide a useful tool for understanding the molecular mechanisms of inflammatory skin disorders in which TGFβ1 is overexpressed.


INTRODUCTION

Psoriasis is a common inflammatory skin disease that has a severe negative impact on a patient’s quality of life and can be an economic burden. Histologically, psoriasis is characterized by epidermal hyperplasia and parakeratosis, dilated and prominent blood vessels in the upper dermis, and leukocyte infiltration in the dermis and epidermis (Griffiths and Barker, 2007; Gudjonsson et al., 2007). Psoriatic lesions express high levels of IL-2, IFN-γ, and TNF-α, but not IL-4 and IL-10. Deletion of active T lymphocytes with toxin-coupled IL-2 (DAB389IL-2) or inhibition of migration and activation of T cells with an anti-LFA-1 antibody significantly reduces the severity of psoriasis (Gottlieb et al., 1995; Thaci, 2008). IL-4 or IL-10 therapy also significantly relieves the symptoms of human psoriasis (Ghoreschi et al., 2003; Asadullah et al., 2004). Therefore, psoriasis was widely considered as a Th1 type disease (Sabat et al., 2007) until the discovery of IL-17-producing T cells (Th17); the new findings suggest that Th17 cells have a crucial function in the development of psoriasis (Zaba et al., 2007; Lowes et al., 2008; Di Cesare et al., 2009). Further, a recent study revealed cooperative effects between Th1 and Th17 cells in the pathogenesis of psoriasis (Asadullah et al., 2004; Kyczek et al., 2008).

ALTERATION OF TGFβ1 IN HUMAN PSORIASIS

Transforming growth factor β (TGFβ) is a multipotent cytokine. Among the three isoforms of TGFβ, TGFβ1 is the predominant isoform in the skin. TGFβ binds to type I and type II receptors (TGFβRI and TGFβRII). TGFβRI phosphorylates downstream mediators Smad2 and Smad3. Phosphorylated Smad2 and Smad3 complex with Smad4 to regulate TGFβ-responsive genes (Owens et al., 2008). Expressions of TGFβ receptors and Smads are detected in epidermal keratinocytes (Lange et al., 1999; Quan et al., 2002; Han et al., 2005).

A significant reduction of TGFβ receptors in psoriatic epidermis has been reported (Leivo et al., 1998; Doi et al., 2003). As TGFβ1 is a potent growth inhibitor for keratinocytes, it has been suspected that reduced TGFβ signaling potentiates keratinocyte hyperproliferation in psoriasis epidermis. However, reduced TGFβ receptors could also be a result of an increased TGFβ1 ligand. Increased TGFβ1 in the epidermis and serum has been found in psoriatic patients (Flisiak et al., 2002), and the TGFβ1 serum level correlates with disease severity (Nockowski et al., 2004; Flisiak et al., 2008). Successful treatment resulted in reduced serum levels of TGFβ1 in patients with psoriasis (Flisiak et al., 2003). The mechanism responsible for increased serum levels of TGFβ1 in patients with psoriasis remains unclear; increased TGFβ1 could result from activated stromal cells (Flisiak et al., 2008). On the basis of clinical data, it is difficult to determine whether increased TGFβ1 has a causal function in psoriasis or whether it is simply a consequence of psoriasis pathogenesis.

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Abbreviations: IFN-γ, interferon gamma; IL, interleukin; LFA-1, lymphocyte function-associated antigen 1; MIP, macrophage inflammatory protein; TGFβ, transforming growth factor-β; TGFβRI, type I TGFβ receptor; TGFβRII, type II TGFβ receptor; Th, T helper; TNF-α, tumor necrosis factor alpha

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TGFβ1 OVEREXPRESSION IN KERATINOCYTES INDUCES SKIN INFLAMMATION

In the past, TGFβ1 was considered an anti-inflammatory cytokine with strong immune suppressive effects, as TGFβ1 knockout mice died of autoimmune diseases (Shull et al., 1992; Kulkarni et al., 1993). We developed TGFβ1 transgenic mice, targeting wild-type human TGFβ1 at the epidermis using a keratin 5 (K5) promoter (K5.TGFβ1wt). In this transgenic mouse model, TGFβ1 was overexpressed in the epidermis at levels similar to that of peak expression during cutaneous wound healing (Li et al., 2004). K5.TGFβ1wt transgenic mice surprisingly developed severe inflammatory skin phenotypes, including focal lesions induced by ear tagging, erythematous plaques with a scaly appearance at around 1 month of age, and generalized scaly erythema when skin inflammation progressed. Histologically, K5.TGFβ1wt transgenic skin developed significant epidermal hyperplasia and hyperkeratosis with massive inflammatory cell infiltration, neovascularization, and epidermal basement membrane degradation (Li et al., 2004). Epidermal hyperproliferation is mainly a secondary effect of inflammation and angiogenesis, because cultured K5.TGFβ1wt keratinocytes without a stromal component undergo growth arrest (Li et al., 2004). Among infiltrated leukocytes, CD4+ and CD8+ T cells are found in large numbers in the dermis and at the dermal-epidermal junction in K5.TGFβ1wt skin. Molecular analysis showed that Th1 type cytokines predominated in K5.TGFβ1wt skin (Li et al., 2004). The original characterization of this model identified many similarities to psoriasis. However, a recent study shows that despite an increase of this model identified many similarities to psoriasis. To further assess this, we induced TGFβ1 in the epidermis of our gene-switch transgenic mice, in which transgene expression can be regulated by a topical application of RU486 (Lu et al., 2004; Li et al., 2005). When TGFβ1 was induced for 10 days, scaly erythema and plaques developed in transgenic but not in control mouse skin. Histopathology showed that TGFβ1-induced skin recapitulated the pathological alterations observed in K5.TGFβ1wt skin (Figure 1). Continuous RU486 application maintained skin inflammation, and phenotype severity correlated with TGFβ1 expression levels in bigenic mice (data not shown). Leukocyte infiltration found in bigenic skin was similar to that in K5.TGFβ1wt skin. Particularly, CD4 + T cells resided in the dermis, whereas CD8 + T cells predominantly infiltrated the epidermis. Increased BM8+ macrophages and angiogenesis were also prominent in bigenic skin as compared with control skin (data not shown). Interestingly, epidermal thickness and leukocyte infiltration in bigenic skin declined markedly 1 week after withdrawal of RU486 (Figure 1), suggesting that skin inflammation is dependent on TGFβ1 overexpression.

DELETION OF T CELLS DELAYS BUT DOES NOT PREVENT TGFβ1-INDUCED INFLAMMATION

Studies reveal that TGFβ1 is required for naïve mouse CD4+ T cells to differentiate into Th17 cells (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006; Yang et al., 2008). As K5.TGFβ1wt skin showed increased IL17 but did not respond to anti-IL23 treatment (Fitch et al., 2009), it is possible that (1) the Th17-IL23 pathway is not involved in skin inflammation in this model; (2) IL23 may primarily activate Th17 cells, but this function is dispensable with TGFβ1-induced Th17 activation; or (3) Th17 cells initiate but cannot sustain skin inflammation in this model. More thorough studies in the future are needed to delineate these possibilities. To determine whether depletion of all T cells would have a stronger effect in alleviating skin inflammation in K5.TGFβ1wt mice, we crossed K5.TGFβ1wt mice with

![Figure 1. Effects of TGFβ1wt transgene induction in gene-switch-TGFβ1wt skin.](image-url)

The H&E staining of dorsal skin from TGFβ1wt gene-switch mice treated with EOH (i) or RU486 (ii) for 10 days. Epidermal hyperplasia and corneal microabscess (arrow) were noticed on TGFβ1wt induction (ii) and recovered to normal skin after withdrawal of RU486 (iii). The bar in panel (i) represents 100μm for all sections. The dotted line in each section highlights the epidermal-dermal junction.
Figure 2. T-cell depletion delays TGFβ1-induced inflammation. (a) Immunohistochemical staining for T (CD4+) cells, macrophages (F4/80 +), and granulocytes (Ly6G +) in K5.TGFβ1wt/Rag1+/+ and K5.TGFβ1wt/Rag1−/− skin. CD4+ T-cells were largely depleted in K5.TGFβ1wt/Rag1−/− skin. No significant difference in macrophage (F4/80+) and granulocyte (Ly6G+) staining was observed between K5.TGFβ1wt/Rag1+/+ and K5.TGFβ1wt/Rag1−/− skin.

(b) Histological analysis of skins from K5.TGFβ1wt/Rag1+/+ and K5.TGFβ1wt/Rag1−/− mice at 2, 4, and 6 months of age. Skin from K5.TGFβ1wt/Rag1+/+ mice shows profound inflammatory cell infiltration, epidermal hyperplasia, and basement membrane degradation at 2 and 4 months of age, but the phenotype was almost reversed in K5.TGFβ1wt/Rag1−/− mice. Skin from both genotypes showed a similar histological alteration at 6 months of age. The bar in panel (a) and (b) represents 40 μm.
Rag1−/− mice, which lack mature lymphocytes (Mombaerts et al., 1992). Immunohistochemical staining showed that CD4+ T cells were largely depleted in K5.TGFβ1wt.Rag1−/− skin, but total leukocytes (CD45+, data not shown), which are mainly macrophages (F4/80) and granulocytes (Ly6G+) in K5.TGFβ1wt.Rag1−/− skin, were similar to K5.TGFβ1wt.Rag1+/− skin (Figure 2a). This finding suggests that overall leukocyte infiltration was not a secondary event of T-cell activation, but rather a direct chemotactic effect of TGFβ1 in this model. Histologically, a significant reduction in epidermal hyperplasia and inflammation has been observed as early as 3 weeks in K5.TGFβ1wt.Rag1−/− compared with K5.TGFβ1wt.Rag1+/− littermates, and this effect is sustained over 4 months (Figure 2b). In accordance with phenotype changes, the expression levels of pro-inflammatory cytokines, including TNF-α and IL-1β in skin as determined by quantitative RT-PCR, were significantly decreased in K5.TGFβ1wt.Rag1−/− skin when compared with K5.TGFβ1wt.Rag1+/− skin (data not shown). The anti-inflammatory effect of T-cell depletion was gradually lost after 6 months of age in K5.TGFβ1wt/Rag1−/− mice (Figure 2b). These data suggest that T cells are important drivers of TGFβ1-mediated skin inflammation, especially during the early stages of inflammation. However, accumulated pro-inflammatory cytokines or chemokines from other inflammatory cells may be crucial in maintaining TGFβ1-mediated skin inflammation.

THERAPEUTIC APPROACHES FOR PSORIASIS REDUCED INFLAMMATION IN K5.TGFβ1WT SKIN

As the phenotypes of K5.TGFβ1wt mice are more similar to psoriasis than to any other inflammatory skin diseases, we wondered whether current therapies for psoriasis would alleviate K5.TGFβ1wt phenotypes. We first tested the efficacy of Enbrel (Etanercept), a soluble fusion protein composed of TNF-α receptors and the Fc portion of human IgG1, which competitively binds to TNF-α and prevents TNF-α from binding to endogenous receptors (Zeichner and Lebwohl, 2007). We treated K5.TGFβ1wt mice with Enbrel at 6 weeks of age, when skin inflammation began to develop. Enbrel was injected intraperitoneally to K5.TGFβ1wt mice at a dosage of 0.4 mg per mouse every other day for up to 6 weeks; controls were treated with normal saline. Beginning 3 weeks after Enbrel treatment, K5.TGFβ1wt mice showed a reduction of epidermal hyperplasia and fewer numbers of infiltrated T cells compared with control mice (Figure 3b). Improvements in the gross appearance of Enbrel-treated mice were obvious after 6 weeks of treatment (Figure 3a and b). Without treatment, the phenotype worsened. In contrast, 6 weeks after Enbrel treatment, K5.TGFβ1wt mice exhibited few scaly plaques or mild skin inflammation. Histology shows that the treated skin exhibited a marked reduction in epidermal hyperplasia (Figure 3b).

Second, we tested the efficacy of Rosiglitazone (Avandia) on K5.TGFβ1wt mice. Avandia, a peroxisome proliferator-activated receptor-γ agonist used to treat type II diabetes, is also used to treat psoriasis (Pershadsingh, 2004). We administered Avandia 0.04 mg ml−1 through drinking water in K5.TGFβ1wt mice or RU486-treated gene-switch TGFβ1 mice when skin inflammation was well developed, starting from when they were 2–3 months old up to 1 year of age. Transgenic littermates that received no Avandia in drinking water were used as controls. Terminal differentiation markers of the epidermis, loricrin and fillagrin, which were lost in TGFβ1wt skin, were restored after only 3 weeks of Avandia treatment (Figure 4a). Total (CD45+) leukocytes and CD4+ lymphocytes were reduced, whereas BM8+ macrophages were slightly reduced in TGFβ1wt skin 3 weeks after Avandia treatment (Figure 4b). With an 8-week treatment, lesions on treated transgenic mice were much less severe than in non-treated mice, and epidermal hyperplasia was appreciatively reduced (Figure 5a). Interestingly, at this stage, in addition to a considerable reduction of T cells and leukocytes in the lesion (data not shown), the number of macrophages stained by BM8 was significantly decreased in the skin of K5.TGFβ1wt mice (Figure 5b). These data further suggest that activated macrophages contribute significantly to the maintenance of TGFβ1-mediated skin inflammation. It should be

![Image](320x560 to 549x697)

![Image](324x434 to 545x537)

**Figure 3.** Etanercept therapy relieves the inflammatory symptoms of K5.TGFβ1wt mice. (a) Typical gross appearances of K5.TGFβ1wt mice before and after Enbrel treatment for 6 weeks. Normal saline-treated mice were used as controls. Minor skin inflammation appeared on the ear of K5.TGFβ1wt mice at the age of 6 weeks (i & iii) and progressed to spread over most of the body area with scaly plaques or skin inflammation at the age of 12 weeks (ii). Enbrel therapy prevented the acceleration of skin phenotypes (iv). Arrows point to inflammation sites. (b) H&E staining of K5.TGFβ1wt skin sections at 6 weeks with and without Enbrel treatment (left) revealed a significant reduction of epidermal thickness. K5.TGFβ1wt skin exhibited an alleviative infiltration of CD4 and CD8 T cells and a mild reduction in epidermal thickness as early as 3 weeks after starting Enbrel therapy (middle and right panels). The bar in panel (b) represents 40 μm.
noted that mRNA expression levels of many pro-inflammatory molecules, for example, TNF-α, IL-1α, IL-1β, IL-2, IL-6, and MIP-2 (IL-8 homolog), were markedly reduced after an 8-week treatment with Avandia (Figure 5c). As many of these inflammatory cytokines can be produced in multiple cell types, including inflammatory cells, keratinocytes, and fibroblasts, it is likely that Avandia targets multiple cell populations, which seems to be more effective than depleting only T cells. Indeed, the anti-inflammatory effect of Avandia in K5.TGFβ1wt mice persisted during treatment for up to 1 year of observation.

**SUMMARY**

Although many of the pathological and molecular alterations in K5.TGFβ1wt mice are more similar to psoriasis than to any other inflammatory skin diseases, there are differences. First, the lack of a major growth factor targeted at the K5.TGFβ1wt epidermis accounts for less epidermal hyperproliferation than in human psoriasis or mouse psoriasis models expressing a potent growth factor, for example, in Stat3 or amphiregulin transgenic mice (Cook et al., 2004; Sano et al., 2005). Second, the role of activated T cells in skin inflammation is limited to the initiation stage in K5.TGFβ1wt mice. Third, anti-IL23 treatment, which is an effective psoriasis therapy, does not alleviate skin inflammation in K5.TGFβ1wt mice. Finally, pathological alterations similar to atopic dermatitis are also reported in these mice (Fitch et al., 2009). As molecular and
pathological alterations similar to psoriasis occur much earlier (3 weeks) (Li et al., 2004) than in atopic dermatitis (4-6 months) (Fitch et al., 2009), environmental factors contributing to the latter pathological alterations remain to be examined. Similar to all other psoriasis-like mouse models, it is a challenge to make a humanized psoriasis mouse model because of significant differences in skin anatomy and immune system between the two species and the complex nature of psoriasis. On the basis of current findings, it seems that TGFβ1 overexpression alone is insufficient to mimic all characteristics of psoriasis. Conversely, TGFβ1 overexpression may be a contributing factor in several inflammatory skin disorders. An examination of changes in TGFβ signaling among different inflammatory skin diseases will help delineate the conditions in which TGFβ signaling has a pathological role. Additional studies from multiple laboratories using our K5.TGFβ1null mice will provide a more comprehensive understanding of the role of TGFβ1 overexpression in inflammatory skin diseases.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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REFERENCES


