Transgenic Expression of Interleukin-13 in the Skin Induces a Pruritic Dermatitis and Skin Remodeling

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IL-13 has been implicated in the pathogenesis of allergic diseases, including atopic dermatitis (AD). However, a direct role of IL-13 in AD has not been established. We aimed to develop an inducible transgenic model in which IL-13 can be expressed in the skin and to define the resulting dermal phenotype and mechanisms involved. The keratin 5 promoter was used with a tetracycline-inducible system to target IL-13 to the skin. The clinical manifestations, dermal histology, cytokine gene regulation, and systemic immune responses in the transgenic mice were assessed. IL-13 was produced exclusively in the skin and caused a chronic inflammatory phenotype characterized by xerosis and pruritic eczematous lesions; dermal infiltration of CD4+ T cells, mast cells, eosinophils, macrophages, and Langerhans cells; upregulation of chemokine and cytokine genes, including thymic stromal lymphopoietin; and skin remodeling with fibrosis and increased vasculature. The dermal phenotype was accompanied by elevated serum total IgE and IgG1 and increased production of IL-4 and IL-13 by CD4+ cells from lymphoid tissues and peripheral blood mononuclear cells. IL-13 is a potent stimulator of dermal inflammation and remodeling and this transgenic model of AD is a good tool for investigating the underlying mechanisms in the pathogenesis of AD.

Journal of Investigative Dermatology (2009) **129,** 742–751; doi:10.1038/jid.2008.295; published online 2 October 2008

INTRODUCTION

Atopic dermatitis (AD) is a pruritic, chronic inflammatory skin disease that affects 15–20% of children and 1–3% of adults. The prevalence of AD has been steadily increasing during the past three decades (Leung *et al.*, 2004). Th2-dominated immune responses are believed to contribute to the pathogenesis of AD, particularly in the early stage. Analysis of biopsies of affected skin of AD patients revealed increased numbers of Th2 cells expressing IL-4 and IL-13 mRNA (Hamid *et al.*, 1994; Novak *et al.*, 2003). IL-4 and IL-13 are important Th2 cytokines.

IL-13 has some effects in Th2 inflammation that are key features in both asthma and AD. The effects include the ability to induce IgE production (Emson *et al.*, 1998), CD23 expression (Zurawski and de Vries, 1994), endothelial P-selectin and vascular cell adhesion molecule-1 expression (Bochner *et al.*, 1995; Woltmann *et al.*, 2000), and inhibition of eosinophil apoptosis (Horie *et al.*, 1997). Overexpression of IL-13 in the murine lung causes eosinophil, lymphocyte and macrophage-rich inflammation, mucus metaplasia, and airway hyperresponsiveness on methacholine challenge (Zhu *et al.*, 1999). In AD, memory T cells expressing the skin homing receptor, cutaneous lymphocyte-associated antigen, produced increased levels of IL-13 (Akdis *et al.*, 1997). IL-13-stimulated keratinocytes preferentially attract CD4+CCR4+ T lymphocytes (Purwar *et al.*, 2006). IL-13 also downregulates antimicrobial peptide expression in eczematous skin, which may account for their propensity toward recurrent skin infections (Ong *et al.*, 2002; Nomura *et al.*, 2003).

These studies have established a close relationship between IL-13 and AD. However, neither the direct effects of IL-13 on dermal tissue nor the effector functions of IL-13 in the skin have been addressed. We hypothesized that IL-13 alone is sufficient to initiate inflammatory responses in the skin that mimic AD. In this study we developed an externally regulatable overexpression transgenic model system in which IL-13 is selectively expressed in murine skin. Analyses show that expression of IL-13 in the skin resulted in a chronic pruritic inflammatory phenotype with many characteristics closely resembling those of human AD. Many proinflammatory cytokines, including thymic stromal lymphopoietin (TSLP), were highly upregulated in the skin and the dermal phenotype was accompanied by a systemic Th2-prone environment.

RESULTS

Generation of transgenic mice

To obtain mice that express IL-13 specifically in the skin we first generated mice carrying the transgene TRE-Tight-IL-13

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Abbreviations: AD, atopic dermatitis; Dox, doxycycline; IHC, immunohistochemistry; K5, keratin 5; MMP-9, matrix metalloproteinase-9; PBMC, peripheral blood mononuclear cell; Tg, transgenic; TGF-β1, transforming growth factor-β1; TSLP, thymic stromal lymphopoietin; VEGF, vascular endothelial growth factor

Received 10 June 2008; revised 30 July 2008; accepted 31 July 2008; published online 2 October 2008

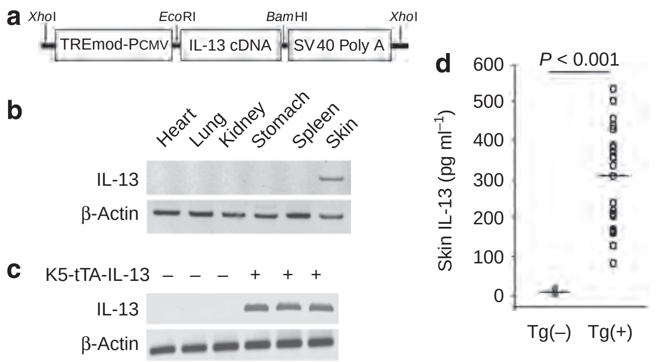

Figure 1. **Targeting IL-13 to the skin.** (**a**) Schematic construct of TRE-Tight-IL-13. (**b**) Tissue specificity of IL-13 expression in Tg(+) mice as determined by RT–PCR. The experiments were started by withdrawing Dox and total RNA from different tissues was analyzed for IL-13 mRNA expression. (**c**) IL-13 mRNA expression in the skin of Tg(+) mice in comparison with Tg(–) mice 6 weeks off Dox. (**d**) IL-13 protein in the skin extract 8 weeks after Dox withdrawal ($n=36$, each group).

(Figure 1a) and crossbred these mice with the K5-tTA mouse line (Diamond *et al.*, 2000). The offspring that carried both transgenes were designated as K5-tTA-IL-13 mice (or Tg(+) mice) and were used in experiments in comparison with Tg(–) littermate control mice.

Inducible expression of IL-13 in the skin

We then tested if expression of IL-13 could be induced by doxycycline (Dox) and was restricted to the skin. The keratin 5 (K5) promoter controlled tTA is inactive in the presence of Dox and active in the absence of Dox. To express IL-13 only in the adult mice, we gave Dox (1 mg ml^{-1} with 4% sucrose) in the drinking water of the K5-tTA-IL-13 mice and their Tg(–) littermates until they were at least 6 weeks of age. In the beginning of each experiment, Dox was withdrawn and the induction of the IL-13 transgene was initiated.

IL-13 was not detected at either the mRNA or protein level in any tissues of Tg(–) mice or in Tg(+) mice with Dox suppression (data not shown). However, after Dox withdrawal for 6 weeks IL-13 mRNA was readily detected in the skin but not in other tissues of Tg(+) mice (Figures 1b and c). Similarly, IL-13 protein was readily detected in the skin extracts of Tg(+) mice after induction (Figure 1d). All subsequent experiments were performed to compare Tg(+) mice and Tg(–) littermate controls without Dox starting from 6 weeks of age. Immunohistochemistry (IHC) showed that IL-13 was expressed in the keratinocytes, with less staining in the dermal layer, indicating that IL-13 was produced by keratinocytes (data not shown). These results demonstrated that using the K5-tTA system, we targeted the IL-13 transgene to the skin in an inducible fashion.

IL-13-induced inflammation in the skin

Gross and histological examination showed no abnormalities in the skin of Tg(–) mice with or without Dox, indicating that Dox had no effect on the skin. Similarly, Tg(+) mice given Dox did not show any sign of inflammation because no IL-13 was expressed under this condition (Figure 2a). In contrast, 6–8 weeks after Dox withdrawal, Tg(+) mice developed pruritus (indicated by constant and intensive scratching of the affected areas), loss of hair, erythema, crusting, excoriation, bacterial pyoderma, and erosions in the skin, mostly involving the back and abdominal areas. With continued IL-13 induction, the lesions progressed and became more extensive, including dry lichenified skin lesions (Figure 2a). Quantitative evaluations of the mice using the criteria described in Materials and Methods showed that Tg(+) mice with the IL-13 transgene turned on had increased clinical scores over time (Figure 2a). Histologically, in comparison to the Tg(–) littermates, the lesional skin from Tg(+) mice showed thickening of the epidermal and dermal layers, spongiosis, hyperkeratosis, and marked cellular infiltration (Figure 2b). The inflammatory infiltration was characterized by increased numbers of mononuclear cells and eosinophils in the subepidermal, intradermal, and perivascular spaces whereas neutrophils were rarely seen (Figure 2b). To further define the cell types, skin samples were stained using antibodies to specific markers for eosinophils, CD4+ cells, and F4/80+ cells, including activated macrophages and Langerhans cells, which are important cell types that contribute to the inflammatory response in human AD (Leung *et al.*, 2004). Significantly increased accumulation of major basic protein+ eosinophils (Figure 2c) and F4/80+ cells (Figure 2d) was seen in the dermal layer of the skin of Tg(+) mice as compared to the Tg(–) controls. Similarly, in the lesional skin of Tg(+) animals increased numbers of CD4$^+$ cells were found (Figure 2e). Quantitative evaluation of these cell types is shown in Table 1. These findings demonstrated that expression of IL-13 in the skin resulted in a chronic pruritic inflammatory skin phenotype with many characteristic changes closely resembling eczematous inflammation observed in human AD.

IL-13-induced increased mast cells and mediators

Mast cells and their mediators contribute to the pathogenesis of human AD. Increased numbers of mast cells were found in the skin of AD patients. Studies were undertaken using toluidine blue staining to detect mast cells in the skin of K5-tTA-IL-13 mice and their littermate controls. Some mast cells were present in the skin of Tg(–) mice. However, IL-13 caused a fourfold increase in the numbers of mast cells in the lesional and nonlesional skin of Tg(+) animals (Figures 3a and b). This was associated with increased tissue histamine and β-hexosaminidase in the skin of Tg(+) mice as compared with those in Tg(–) mice (Figures 3b and c).

IL-13-induced upregulation of chemokine and cytokine expression in the skin

To understand the mechanisms by which IL-13 induced skin inflammation, we examined the expression of selected chemokines in Tg(+) and Tg(–) mice. In the skin of Tg(+) mice, the levels of mRNA encoding CCL11/Eotaxin, CCL17/TARC, CCL27/CTACK, CCL2/MCP-1, CCL22/MDC, and CCL5/RANTES were highly increased compared with those in the skin of Tg(–) mice (Figure 4), indicating that IL-13 is a potent stimulator of proinflammatory chemokines in the skin.