IL-17A Upregulates Keratin 17 Expression in Keratinocytes through STAT1- and STAT3-Dependent Mechanisms

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Psoriasis, an immunological skin disease, is characterized by epidermal hyperproliferation, chronic inflammation, and an accumulation of infiltrating T cells. IL-17A is a key cytokine that has a critical role in the pathogenesis of psoriasis. Keratin 17 (K17) is strongly expressed in psoriatic lesions but not in normal skin. Thus, K17 expression is regarded as a hallmark of psoriasis. We previously reported that the K17/T cells/cytokine autoimmune loop was involved in psoriasis. However, the relationship between IL-17A and K17 has yet to be determined. In the present study, IL-17A-induced K17 expression was confirmed in cultured keratinocytes in both mRNA and protein levels. In addition, increased K17 expression was found in the epidermis of IL-17A-injected mouse skin. The regulatory mechanism of K17 expression was further investigated. We found that both the signal transducer and activator of transcription (STAT) 1 and STAT3 pathways were involved in the upregulation of K17 expression induced by IL-17A, and that such regulation could be partially suppressed by STAT1 or STAT3 small interfering RNA and inhibitor. Our data suggest that IL-17A can upregulate K17 expression in keratinocytes in a dose-dependent manner through STAT1- and STAT3-dependent mechanisms. The results indicate that IL-17A might be an important cytokine in the K17/T cells/cytokine autoimmune loop associated with psoriasis.

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INTRODUCTION

Keratin 17 (K17), a myoepithelial keratin, is overexpressed in psoriatic lesions, and is not found in healthy epidermis. Hence, K17 is considered to be a hallmark of psoriasis (de Jong *et al.*, 1991). It has been shown that IFN-γ can upregulate K17 expression by activating signal transducer and activator of transcription (STAT) 1, a transcription factor (Jiang *et al.*, 1994). K17 may function as an autoantigen in the immunopathogenesis of psoriasis, which may be a major target for autoreactive T cells (Fierlbeck *et al.*, 1990). Some restricted T-cell epitope regions, found on the K17 molecule, can promote the proliferation of psoriatic T cells and induce the production of IFN-γ effectively (Shen *et al.*, 2005). Thus, a positive-feedback mechanism, previously described as a K17/T cell/cytokine autoimmune loop, may exist to drive the pathogenesis of psoriasis (Bockelmann *et al.*, 2005; Shen *et al.*, 2006). Recently, the relationship between K17 overexpression and psoriasis has captured the attention of dermatologists, but the regulation and biological roles of K17 in psoriasis remains unknown.

Psoriasis is now believed to be a mixed Th1/Th17 cell-mediated autoimmune disease, in which the likely induction of IFN-$\gamma^{(+)}$ IL-$17^{(+)}$ cells is considered to be pathogenic (Ksatelan *et al.*, 2004; Arican *et al.*, 2005). IL-17A is a cytokine produced by Th17 cells that helps to recruit neutrophils and drive inflammatory responses (Albanesi *et al.*, 2000; Weaver *et al.*, 2007). IL-17A expression is detectable in psoriatic skin lesions and allergic contact dermatitis, but not in normal skin (Teunissen *et al.*, 1998; Albanesi *et al.*, 1999). Overexpression of IL-17A at both gene transcript and protein levels has been observed in serum and skin lesions of psoriatic patients, and is correlated with the severity of the disease (Arican *et al.*, 2005; Caproni *et al.*, 2009; Kagami *et al.*, 2010). This evidence strongly suggests that IL-17A is involved in the pathophysiology of psoriasis (Fitch *et al.*, 2007; van Beelen *et al.*, 2007).

On the basis of two previously reported findings, (1) that the K17/T cells/cytokine loop functions in the pathogenesis of psoriasis (Shen *et al.*, 2006), and (2) that IL-17A is a Th17-produced cytokine that activates three signaling pathways, which is detectable in psoriatic skin lesions, we hypothesized that IL-17A may be a key cytokine member of the K17/T cells/cytokine autoimmune loop and induce K17 expression by activating signaling pathways, and therefore participate in the

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Abbreviations: K17, keratin 17; NHEK, normal human epidermal keratinocyte; siRNA, small interfering RNA; STAT, signal transducer and activator of transcription

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development of psoriasis. We tested this hypothesis in the present study by observing the effect of IL-17A on the expression of K17 in HaCaT human keratinocytes, normal human epidermal keratinocytes (NHEKs), and mice model.

RESULTS

The upregulation of K17 expression in IL-17A-induced keratinocytes

To determine whether IL-17A can upregulate K17 expression, we pretreated HaCaT cells with IL-17A in different concentrations (10, 50, 250, or 500 U ml^{-1}), and then analyzed K17 mRNA levels with real-time PCR. As expected, we found that K17 mRNA levels increased with IL-17A concentration in a dose-dependent manner, especially at higher concentrations (250 and 500 U ml^{-1}), as compared with the levels in untreated cells (Figure 1a). Meanwhile, we constructed two reporter vectors containing different regions of K17 promoter spanning −244 to +18 bp (ΔK17p1) and −686 to +18 bp (ΔK17p2). IL-17A upregulated the activity of ΔK17p2 promoter, whereas ΔK17p1 did not respond to IL-17A, suggesting that the element in response to IL-17A is located between −686 and −244 bp of the K17 promoter (Figure 1b). To further confirm this finding, ELISA and western blot assays were performed to measure K17 protein expression. K17 protein expression was upregulated by IL-17A at concentrations of 250 U ml^{-1} or higher. However, no significant difference in the expression levels of K17 protein was

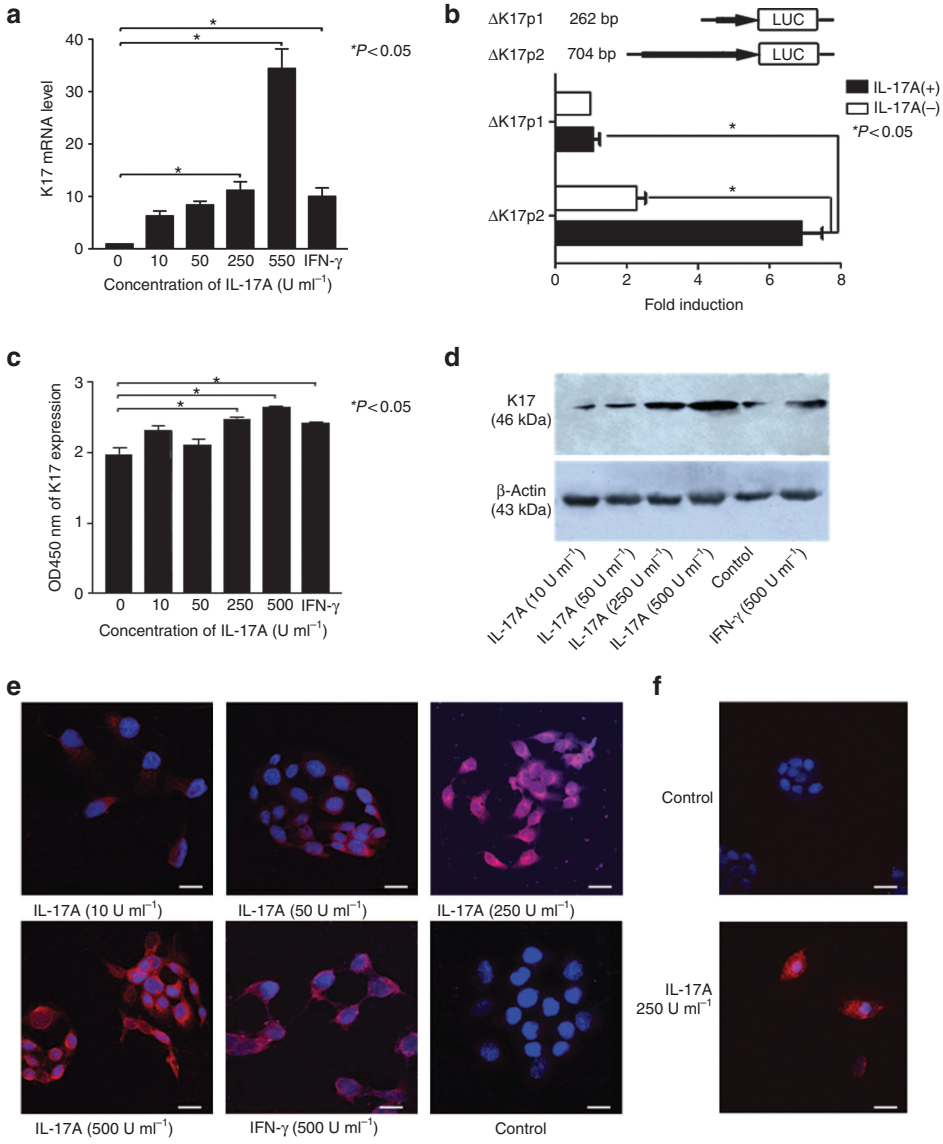

Figure 1. Upregulation of keratin 17 (K17) expression in IL-17A-induced keratinocytes. (**a**) Real-time PCR analysis of K17 mRNA level. Data were expressed as $2^{-\Delta\Delta CT}$ relative to untreated HaCaT cells. (**b**) K17 promoter activity was examined via reporter assay under the stimulation of IL-17A (250 U ml^{-1}). Filled arrows represent the K17 promoter region, the box the firefly luciferase gene. (**c**) ELISA analysis of K17 expression. (**d**) Protein expression of K17 was examined using western blot. (**e**) Immunofluorescence was performed on HaCaT cells to measure K17 expression. 4′-6-Diamidino-2-phenylindole staining for nuclei is blue. (**f**) Immunofluorescence was performed on normal human epidermal keratinocytes to measure K17 expression. Bar = 30 μm. Results represent mean ± SEM from three independent experiments. *P<0.05 was considered significant.