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Detection of IL-20 and its receptors on psoriatic skin

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Abstract

The aim of this study was to investigate the expression of interleukin-20 (IL-20) and its receptors on psoriatic skin by immunohistochemical analysis and to evaluate the correlation of CD8-positive T lymphocytes with epidermal proliferation. Overexpression of IL-20 and its receptors was detected in the keratinocytes of the lesional skin of psoriasis and spongiotic dermatitis. The expression pattern of IL-20 spreads throughout the whole layer of epidermis, while IL-19 was expressed in up to three or four layers suprabasally. The serum level of IL-20 in psoriatic patients was significantly lower than that in healthy controls. IL-20 upregulated KGF transcripts on CD8-positive T cells. We hypothesize that overexpression of IL-20 is correlated with keratinocyte proliferation that acts through their receptor complex expressed by keratinocytes themselves. Furthermore, IL-20 can stimulate CD8-positive lymphocytes to produce KGF, which may contribute to sustaining the hyperproliferative status of the keratinocytes.

Keywords: Interleukin-20; Interleukin-20 receptors; Psoriasis; Keratinocytes; Keratinocyte growth factor

Introduction

Psoriasis is an inflammatory dermatosis characterized by abnormal epidermal proliferation. Previous studies indicate that T cells and cytokines are of major importance in the pathophysiology of this immune-mediated disorder [1–4]. Psoriasis exhibits cutaneous and systemic overexpression of several proinflammatory cytokines, particularly type-1 cytokines (e.g., IL-2, -6, -8, and -12; IFN-γ; and TNF-α, overexpression of which is responsible for the initiation, maintenance, and recurrence of skin lesions) [2]. A pivotal role of T cells in pathogenesis of psoriasis has been demonstrated by the presence of activated T cells in psoriatic plaques as well as by the efficacy of inhibitory reagents for T cell in psoriasis treatment [3].

IL-20 is identified as a member of the IL-10 family [4], which includes IL-10, -19, -20, -22, -24 (MDA-7), and -26 (AK155). Despite a partial homology in their amino acid sequences, they have diverse biological functions. IL-19, IL-20, and IL-24 share receptor complexes; all three are capable of signaling through the IL-

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20R1/IL-20R2 heterodimer, and both IL-20 and -24 can also use IL-22R and IL-20R2 [5]. However, the main biological effects of these three cytokines appear quite distinct: immune activity with IL-19 [6,7], skin biology with IL-20 [4], and tumor apoptosis with IL-24 [8]. IL-20 binds to its receptor on keratinocytes and stimulates a STAT3-containing signal-transduction pathway. Recent studies have suggested pathogenic roles of IL-19 and -20 in psoriasis. Overexpression of IL-20 in transgenic mice causes neonatal lethality with psoriasis-like skin abnormalities, including hyperkeratosis, thickened epidermis, and aberrant epidermal differentiation [4]. Moreover, IL-20 receptor mRNA is markedly upregulated in human psoriatic skin compared to normal skin. Overexpression of IL-19 mRNA in psoriatic skin was recently demonstrated [9]. IL-19 signals through the IL-20R1/IL-20R2 heterodimer [5]. We previously demonstrated that IL-19 could stimulate monocytes to produce IL-6 and TNF-α [7], two leading effector cytokines in the pathogenesis of psoriasis, and it was also associated with psoriasis [10]. The biological functions of IL-20 in keratinocyte proliferation remain unclear.

Previous investigations of IL-19 and -20 in psoriasis are mainly focused on the expression level of mRNA. Whether the protein level of IL-20 and its receptor was also upregulated in psoriasis has not yet been demonstrated. To explore the role of IL-20 and the IL-20 receptors in psoriasis, we developed antibodies against human IL-20 and the IL-20 receptors to study the expression level and pattern of these proteins. Immunostaining for IL-20, IL-20R1, and IL-20R2 was performed in skin sections from psoriasis patients. Specimens from healthy skin and spongiotic dermatitis were stained as controls. One pair of monoclonal antibodies against IL-20 was used in an ELISA system to detect the serum level of IL-20 in psoriasis patients and healthy controls. Since T cells play a key pathogenic role in psoriasis, we also treated CD8-positive T cells with IL-20 to examine whether there was any altered expression level of keratinocyte growth factor (KGF).

Methods

Participants

Forty-two patients with psoriasis and two patients with spongiotic dermatitis from the dermatology departments of Chi-mei Medical Center and National Cheng Kung University Hospital in Tainan and from Chang Gung Memorial Hospital and Veteran General Hospital in Kaohsiung, Taiwan and forty-two healthy controls were enrolled in this study. The patients had moderate to severe psoriasis vulgaris (PASI score: 8–24). Biopsies and blood samples were taken after obtaining informed consents from all participants. The study was approved by the Human Research Review Committees of the four hospitals and was performed in accordance with the Declaration of Helsinki Guidelines.

Expression and purification of human IL-19, IL-20, and the extracellular domain of human IL-20R2 recombinant proteins

Human IL-19 recombinant protein was expressed and purified as previously described [11]. A cDNA clone coded for the human IL-20 sequences from Leu to Leu (amino acids 25 to 176) was inserted into pET15 (Novagen, Madison, WI). The protein was expressed primarily in the inclusion body and purified to >95% using metal chelation chromatography and refolding. This protein was used in biological function analysis in vitro and for the generation of monoclonal antibody (described in the following paragraph). RNA was isolated from HaCaT cells and reverse transcribed into cDNA. The extracellular domain of IL-20R2 was amplified with PCR, and the amplified PCR fragment coded from Asp to Pro (amino acids 30 to 232) was inserted into the expression vector of *Pichia pastoris* (pPICZ-αA; Invitrogen, Inc., San Diego, CA). A Tag consisting of six histidine residues was placed at the C-terminus of the recombinant proteins. IL-20R2 extracellular domain protein was expressed and purified from the culture media of the *P. pastoris* yeast cells using metal affinity chromatography. This protein was used for the generation of polyclonal antibody (described below).

Generation of mouse monoclonal antibodies against human IL-19 and IL-20

Monoclonal antibodies against IL-19 were generated as previously described [11]. Selection and characterization of monoclonal antibodies against human IL-20 were based on the standard protocols. BALB/cJ mice were immunized subcutaneously every week for 4 weeks with recombinant human (h)IL-20 protein (100 μg/mouse) emulsified with an equal volume of Freund's complete/incomplete adjuvant. Three days before fusion, three mice were boosted with an intravenous injection of the antigen without adjuvant. Spleen cells (1.2×10^8) from immunized mice were fused with X63-Ag8-6.5.3 myeloma cells (1.5×10^7) with PEG 4000 (Merck and Co., Inc., Whitehouse Station, NJ). After fusion, the cells were distributed into 96-well plates and cultured in HAT medium for 14 days. Using ELISA, culture supernatant was tested for antibody reacting with hIL-20. To clone the selected hybridoma cells, the limiting dilution was carried out twice. The hybridoma cells were cultured in Dulbecco's Modified Eagle medium (GIBCO, Invitrogen Corporation, Carlsbad, CA) containing 15% fetal calf serum, 1% penicillin/streptomycin, 2% L-glutamine, and 1% adjusted $NaHCO_3$ solution. The isotype of the