Full Length Research Paper

Increased expression of Th17 cytokines in patients with psoriasis

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Psoriasis is a chronic inflammatory skin disease that is thought to be mediated by a new distinct type of T helper cell, called Th17 cells that play an essential pathogenic role in psoriasis. In this study, we measured serum levels of IL-17A and IL-23P19 in 43 psoriatic patients and 30 healthy control using nested real time polymerase reaction chain (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) method, correlating their levels to disease severity, which was calculated by psoriasis area severity index (PASI) score. Serum levels of the studied cytokines were significantly elevated in comparison with normal control serum levels according to determination. Also, serum levels of both IL-17A and IL-23P19 were significantly correlated with PASI score. Our result indicates that Th17 cells might play a key role in the immunopathogenesis of psoriasis and as markers of disease activity.

Key words: Psoriasis, Th17 cell, IL-17A, IL-23P19, real-time quantitative reverse transcription polymerase reaction chain (RT-PCR), enzyme-linked immunosorbent assay (ELISA).

INTRODUCTION

Psoriasis is a complex inflammatory skin disease that affects 1 to 3% of the population worldwide, and kept increasing in recent years (Sabat et al., 2007; Bhalerao and Bowcock, 2001). Psoriasis needs to be treated again and again due to reduplicated recurrences, which cause heavy burden to the patients and their family and seriously influence patient physical and mental health (Krueger, 2002; Arican et al., 2005). Although, the initial events triggering a psoriatic lesion are still unknown, immune, heredity, psychology and environment factors have been shown to play a role in psoriasis pathogenesis (Jin et al., 2008; Yen et al., 2006; Blauvelt, 2008). The current view appreciates the fact that psoriasis results from complex interactions between T cells, dendritic cells (DCs), and keratinocytes (Lowes et al., 2007). Until recently, psoriasis has been considered as a classical type 1 autoimmune disease, with a strong IFN-g T helper 1 (Th1) signal. However, a new subset of T cells, T helper 17 (Th17) cells, has now been described in murine models of autoimmune inflammation (Weaver et al., 2007). Th17 has independent differentiation and growth regulatory mechanism which play an important role in immunological and infectious disease. IL-23P19R, a key factor to Th17 cell, induces the differentiation of naive CD4+T cells into Thl7 that produces IL-17AA (IL-17A), IL-17AF and IL-6. Blocking IL-23P19 or its downstream factors IL-17A and IL-6 can significantly suppress disease development in animal models of antoimmune disease (Weaver et al., 2007).

The identification of Th17 subset has now broadened our understanding of inflammatory process in human disease, which through the production of IL-17A induction of chemokines and recruitment of other effector cells population might have essential function in psoriasis pathogenesis (Ghoreschi et al., 2007). Thus, in this study, the expression of Th17 cytokines level and protein level in serum of the patients with psoriasis was detected by using nested polymerase chain reaction and ELISA respectively, in order to investigate the relationship between the expression of Th17 cytokines and the pathogenesis of psoriasis.

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Table 1. Comparison of expression of IL-17AmRNA between healthy control and patients with psoriasis. Different mark represent significant difference at p<0.05.

Group	n	$\bar{x}$ ± s(copy/µg RNA)	t	P
Patients	35	287172.97 ± 28172.95	57.33*	<0.001
Control	30	10428.14 ± 4335.83	1.22***	<0.001

MATERIALS AND METHODS

Skin biopsies were obtained from 30 healthy controls and 35 patients with psoriasis vulgaris (mean age = 41), which included 19 cases in active stage and 16 cases in resting stage; all were recruited from the skin disease prevention and treatment of Guangzhou. Patients were diagnosed clinically and their disease severity was measured by PASI score. All patients did not receive any topical or systemic therapy for one month prior to the study and also none of our control group subjects had positive family history for psoriasis. Informed written consents were obtained from all subjects, and the study was approved by the Ethics Committee of Huanan Medical University, China.

Serum

4 ml venous blood samples were collected on sterile plane tube and allowed to stand for 30 min at room temperature then centrifuged at 300 g for 5 min. Sera were immediately separated and stored at -20°C until the time of analysis.

RNA isolation

Total RNA was isolated from skin tissue using TRIzol reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's protocol and as described in the online supplement. The tissue was ground with mortar and pestle, cooled by liquid nitrogen of the ground tissue and 100 mg was incubated with 1 mlTRIzol for 5 min at room temperature (RT). Cell debris was removed by centrifugation (12,000 × g at 4°C for 10 min) and 0.4 ml chloroform was added. After vortexing, the mix was incubated for 5 min at RT. The phases were separated by centrifugation (12,000 × g at 4°C for 15 min) and the aqueous phase was transferred to a new tube. 0.6 × volume of isopropyl alcohol and a 0.1 × volume of 3 M sodium acetate were added to this aqueous phase and incubated for 10 min at 4°C. The precipitated RNA was pelleted by centrifugation (12,000 × g at 4°C for 15 min) and after the removal of the supernatant, the RNA was washed twice with 70% ethanol. After drying, the RNA was resuspended in 30 µl DEPC-treated water. The quality and quantity of the RNA was verified by the presence of two discrete electropherogram peaks corresponding to the 28S and 18S rRNA at a ratio approaching 2:1.

Real-time quantitative RT-PCR

In order to detect mRNA expression level of Th17 cytokines, quantitative RT-PCR was performed. Briefly, using mRNA as template, single-stranded cDNAs were generated by Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's directions. Nested PCR experiments were conducted with an ABI Prism 7900 sequence-detection system (Applied Biosystems, Foster City, CA) and SYBR Green PCR Master Mix according to the manufacturer's protocol. The primer sequences of IL-17AA (Genebank Accession NO.NM-022190) were as follows: forward, 5′-GAAGGCAGGAATCACAAT -3′; and reverse, 5′- CCCACGGA-

CACCAGT -3′. The primer sequences of IL-23p19 (Genebank Accession NO. NM_016584) were as follows: forward, 5′-CCGCTTCAAAATCCTTCGC -3′; and reverse, 5′-CCCAAATTTCCCTTCCCATC -3′. GAPDH (Applied Biosystems) served as the internal control. Each sample was normalized on the basis of its GAPDH content. The thermal cycling conditions were as follows: 2 min at 95°C, followed by 40 cycles of 95°C for 15 s and 59°C for 45 s.

ELISA analysis

To detect protein expression of IL-17A and IL-23p19, ELISA was conducted by using assay kit. The human IL-17A ELISA kit (Huamei, china) is an *in vitro* ELISA for the quantitative measurement of human IL-17A in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human IL-17A coated on a 96-well plate. Standards and samples were pipetted into the wells and IL-17A present in a sample was bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-human IL-17A antibody was added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution was added to the wells and color developed in proportion to the amount of IL-17A bound. The stop solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm RayBiotech, Inc.

The human IL-23p19 ELISA kit (Huamei, china) is a quantitative measurement of human IL-23p19 cell lysate and tissue lysate. The IL-23p19 was detected according to the manufacturer's protocol.

Statistics analysis

To calculate the statistical differences between the control and patients with psoriasis, the statistical package SPSS13.0 (SPSS Incorporated, Chicago) was used for all analysis. One-way ANOVA test was used to determine the significance of differences among the groups. All values were expressed as mean ± SD. In general, p values less than 0.05 were considered statistically significant.

RESULTS

IL-17A and IL-23P19 mRNA expression in lesional psoriatic skin and normal skin

As shown in Table 1, the expression of IL-17A mRNA was higher in lesional skin of psoriasis than in normal skin. Table 2 shows the results of IL-23p19 level in lesional psoriatic skin and normal skin. Specific transcripts for IL-23p19 were detected in lesional psoriatic skin and normal skin. The expression of IL-23p19 mRNA was increased in all lesional skin biopsies of patients with psoriasis as compared to that in normal

Table 2. Comparison of expression of IL-23P19 mRNA between healthy control and patients with psoriasis.

Group	n	$\bar{x}$ ± s(copy/μg RNA)	t	P
Patients	35	474342.21 ± 35734.04	6.91*	<0.001
Control	30	99813.45 ± 9286.91	0.461***	<0.001

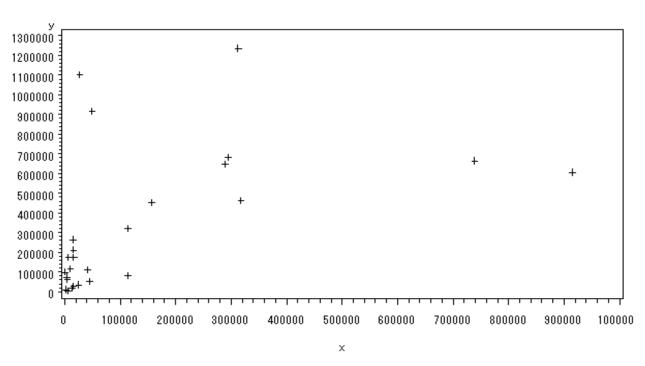Different mark represent significant difference at p<0.05.

Figure 1. Correction between expression of IL-17A mRNA and IL-23P19 mRNA.

skin.

Correlation between mRNA levels of IL-17A and IL-23P19

The mean mRNA levels of IL-17A in psoriatic patient was found to be significantly correlated to mRNA levels of IL-23p19 ($r = 0.469$, $P<0.05$) as shown in Figure 1, while mRNA level of IL-23p19 and IL-17A were not correlated to PASI score ($r = 0.224$, $P = 0.274$).

Serum levels of IL-17A and IL-23P19 among cases and control

Serum levels of IL-17A and IL-23p19 were increased in all rest stage and all active stage of patients with psoriasis as compared with that in healthy control. Meanwhile, the serum levels of IL-17A and IL-23p19 in all active stage was higher than that of the rest stage. All serum cytokine levels are illustrated in Table 3. In addition, the mean serum levels of IL-17A and IL-23p19 in psoriatic patient was found to be significantly correlated to disease severity index PASI of *Psoriasis vulgaris* ($r = 0.732$, 0.611, $P<0.05$). In addition, the mean serum levels of IL-17A and IL-23p19 in psoriatic patient found to be significantly correlated to disease severity index PASI of psoriasis vulgaris ($r = 0.732$、0.611, $P<0.05$).

DISCUSSION

Psoriasis is a common recurrent inflammatory skin disease, characterized by hyperproliferative epidermis and cutaneous lymphocyte infiltrate. The cause of psoriasis is still unknown and because psoriasis affects the epidermis, it is regarded as an epidermal disease (Lowes et al., 2007). T-cells involved in psoriasis pathogenesis were initially thought to be Th1 differentiated due to the presence of elevated level of IFN-γ. However, the recent discovery of Th17 cell and its potential involvement in psoriasis generate more complexity to the disease

Table 3. Comparison of serum levels of IL-17A and IL-23p19 between different groups.

Group	n	IL-17A	IL-23P19
Control	30	48.50±20.67*	110.10±49.67*
Active stage	19	210.46±24.57	281.72±37.06
Rest stage	16	127.59±32.69	156.23±46.18
F		38.56	42.27
P		<0.001	<0.001

Different mark represent significant difference at p<0.05.

(Barker et al., 1991, Szabo et al., 1998, Uyemura et al., 1993). Several studies has reclassified psoriasis as Th17 disease (Leonardi et al., 2008; Weaver et al et al., 2007; Lowes et al., 2008; Liang et al., 2006; Zheng et al., 2007). Th17 has independent differentiate and growth regulatory mechanism which play an important role in immunological and infectious disease by secretion of IL-17A and IL-6. Previous study demonstrated that IL23 could stimulate the amplification and maintainance of Th17 cell, and the differentiation of Th17 in human bodies depends on IL-Iβ and IL-23P19 (Yen et al., 2006; Blauvelt, 2008). Therefore, IL-23 p19R is a key factor to Th17 cell subpopulation. Recent progress in understanding psoriasis has shown that both local and systemic cytokines collaboratively play a role in psoriasis pathogenesis. Previous studies had identified higher levels of IL-17A and IL-23p19 expression in psoriatic skin lesion (Hideki et al., 2011;Yen et al., 2006; Krueger et al., 2002), which coincided with our data, However, this result seems not to be compatible with the reported result that no increase in IL-17A and IL-23p19 could be detected peripheral in blood (Aician et al., 2005), which may be that our data was obtained form serum not but peripheral blood. Few studies considered their serum levels, thus, this study firstly focused on expression level of IL-17A and IL23p19 in serum by ELISA method, a simple laboratory blood sample method, patient independent, observer independent and accurate marker for cytokine levels detection, while disease severity was calculated by PASI score.

IL-17A plays important direct role in creating proinflammatory and chemotactic environment, enhance IL-6, IL-8 and ICAM-1 expression by keratinocyte, promote lymphocyte infiltration within epidermis, at the same time promote more rapid recruitment of neutrophil through induced chemokine expression (Chan et al., 2007; Liang et al., 2007). Our result demonstrate that the expression of IL-7 and IL-23 was increased in lesional skin biopsies and serum of patients with psoriasis as compared with that of healthy control, and the mean mRNA levels of IL-17A in psoriatic patient was found to be significantly correlated to mRNA levels of IL-23 ($r = 0.469$, $P<0.05$), which confirmed and suggests that IL-23P19 stimulated Th7 is the critical factor in the pathogenesis of psoriasis.

To sum up, our study reveals that the expression of IL-17A and IL-23 was higher in patients with psoriatic than that of healthy control and there were correlation between PASI score and serum level of IL-17A and IL23, indicating that IL-17A and IL-23 in psoriasis was the proximal regulator in its pathogenesis, which may be efficacious to target the activity of IL-23 and IL-1 7 axis for the treatment of psoriasis.

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