Ann Dermatol Vol. 29, No. 4, 2017
pISSN 1013-9087 · eISSN 2005-3894
https://doi.org/10.5021/ad.2017.29.4.422

ORIGINAL ARTICLE

Gene Expression Analysis of Inflammatory Cytokines in Korean Psoriatic Patients

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Background: Although phenotypic heterogeneity of psoriasis is suggested by the alternate activation of either T-helper (Th)1-related or Th17-related cytokines, little is known about the mRNA levels of inflammatory cytokines. **Objective:** To investigate whether there is differential expression of Th1-related and Th17-related inflammatory cytokine genes 1) between psoriatic patients and healthy controls, and 2) between patients with different psoriasis phenotypes. **Methods:** Twenty-five patients with psoriasis (10 with guttate psoriasis and 15 with plaque psoriasis) and 5 healthy volunteers were enrolled in this study. The mRNA levels of circulating cytokines (interleukin [IL]-2, IL-12p40, interferon-γ, IL-17A, IL-22, and IL-23R) were measured by real-time reverse transcription polymerase chain reaction. **Results:** The comparison between psoriatic and healthy control samples revealed that IL-12p40, IL-17A, and IL-22 mRNA levels were significantly higher (approximately 4～6 folds) in the patients with psoriasis. The mRNA levels of these six cytokines in the blood did not differ between the guttate and plaque psoriasis groups. **Conclusion:** We found that the mRNA levels of blood inflammatory cytokines (IL-12p40, IL-17A, and IL-22) were significantly elevated in patients with psoriasis compared to the levels in healthy controls, but they did not significantly differ between patients with guttate and plaque type psoriasis. **(Ann Dermatol 29(4) 422～426, 2017)**

-Keywords-
Cytokines, Gene expression, Psoriasis

INTRODUCTION

Psoriasis is a common chronic inflammatory disorder of the skin, characterized by hyperproliferation of keratinocytes. Although the etiology of psoriasis has not been fully elucidated, it is thought to be associated with a combination of genetic, immunologic, and environmental factors[1]. Psoriasis was originally thought to be associated with a T-helper (Th)1/Th2 cytokine imbalance. Measurement of T cell activity after specific antigen challenge and therapeutic inhibition of activated T cells have been performed in a number of studies[2-4], showing that psoriasis is a T cell-mediated disease. Cytokines in the Th1 pathway include interleukin (IL)-2, IL-12, and interferon (IFN)-γ, whereas the Th17 pathway is primarily mediated by IL-23 and IL-17. IL-22 induces hyperplasia and abnormal differentiation of keratinocytes, thereby playing a key role in the pathogenesis of psoriasis[1,5,6].

Psoriasis is classified into four morphological subtypes (plaque, guttate, pustular, and erythrodermal psoriasis). Plaque-type psoriasis is the most common form (appearing in approximately 90% of patients), and the term 'psoriasis' usually refers to this type of psoriasis[7]. The predominant phenotype, however, may change into other types in the course of disease. For example, chronic plaque psoriasis may transform into inflamed guttate lesions; conversely, guttate psoriasis develops into plaque psoriasis in approximately 70% of patients[8]. The mechanism underlying this change in psoriatic phenotype is not clearly understood, but it may be related to a hyperactive immune pathology

Received September 10, 2016, Revised September 13, 2016, Accepted for publication September 14, 2016

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associated with intercurrent infections, differences in susceptibility or disease-modifying genes[5]. In particular, Christophers[5] proposed that a change in cutaneous inflammation from the IL-12 pathway to the IL-23 pathway, and *vice versa*, affects phenotypes and their stability.

Only a few studies have correlated cytokine levels with psoriasis phenotypes[9-11]. Furthermore, there are a very few studies that have examined gene expression levels of blood inflammatory cytokines in psoriasis cases. Based on the hypothesis that inflammatory cytokine expression varies according to psoriasis phenotypes and that morphological differences indicate distinct potential immunopathogenesis, we measured cytokine mRNA levels in the blood of healthy controls and patients with guttate or plaque psoriasis by real-time reverse transcription polymerase chain reaction (real-time RT-PCR).

MATERIALS AND METHODS

Patients and controls

In this study, 25 patients with psoriasis (10 with guttate psoriasis and 15 with plaque psoriasis) and 5 healthy controls without psoriasis were enrolled. The diagnosis of psoriasis was made clinically and histopathologically. Major inclusion criteria were the following: no local or systemic treatment for at least four weeks prior to enrollment, no significant infection or evidence of immune suppression, and no history of specific medical diagnoses. The guttate group comprised patients whose lesions were either an acute onset or reactivated scattered papules with diameters of <1 cm, whereas the plaque group comprised those with at least one lesion with a long axis of >1 cm.

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and Korean Good Clinical Practices, with the participants' rights and safety taking precedence. Approval for the study was obtained from the institutional review board of The Catholic University of Korea (IRB no. HC15TISI0090). All patients gave their informed consent to participate before screening for inclusion criteria.

Real-time RT-PCR of blood samples

Venous blood samples (10 ml) were collected from the 25 patients with psoriasis and 5 healthy controls into vacuum tubes in sterile conditions.

Total RNA was extracted from 1.5 ml of whole blood using the QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. cDNA synthesis was performed with 5 μl of extracted RNA and the PrimeScript™ 1st strand cDNA Synthesis kit (TaKaRa, Shiga, Japan). PCR was performed with an iTaq™ Universal SYBR® Green Supermix and primers (Bio-Rad, Hercules, CA, USA) targeting interleukins (IL-2, IL-12p40, IFN-γ, IL-17A, IL-22, and IL-23R). The PCR reactions were run on a CFX96 Real-Time Detection System (Bio-Rad), and the PCR thermal cycling steps to amplify the cytokines were the following: a first step of 95°C for 5 min; an amplification cycle (repeated 39 times) of 45/55°C for 30 sec; a melt curve step of 65°C～95°C, with 0.5°C increments at 2～5 sec/step; and a final step of 95°C for 30 sec.

The data presented were normalized to GAPDH (a harmonin-interacting, ankyrin repeat-containing protein and housekeeping gene) mRNA. To determine the relative mRNA expression levels, we used the $\Delta\Delta$Ct method[12]. Primer sequences are shown in Table 1.

Statistical analysis

All data were analyzed using IBM Statistical Package for the Social Sciences (SPSS) ver. 22.0 (IBM Co., Armonk, NY, USA). To examine whether there were any statistically significant differences, nonparametric statistical analyses were performed. To evaluate the differences between two groups (healthy controls vs. patients with psoriasis), we used the Mann-Whitney test. We divided patients into two groups according to psoriasis phenotypes. For comparisons among healthy controls and patients with guttate

Table 1. Primer sequences of all cytokines examined by real-time reverse transcription polymerase chain reaction

	Forward primer	Reverse primer
IL-2	AACTCACCAGGATGCTCACATTTA	TCCCTGGGTCTTAAGTGAAAGTTT
IL-12p40	TGGAGTGCCAGGAGGACAGT	TCTTGGGTGGGTCAGGTTTG
IFN-γ	TCAGCTCTGCATCGTTTTGG	GTTCCATTATCCGCTACATCTGAA
IL-17A	CCACGAAATCCAGGATGCCCAAAT	ATTCCAAGGTGAGGTGGATCGGTT
IL-22	GCTTGACAAGTCCAACTTCCA	GCTCACTCATACTGACTCCGTG
IL-23R	TCAAGAGACACTGATATGTGGAAA	GTAGGTGAGCTTCCCAGCAT
GAPDH	AAGGTGAAGGTCGGAGTCAAC	GGGGTCATTGATGGCAACAATA

IL: interleukin, IFN: interferon.

psoriasis or plaque psoriasis, the Kruskal-Wallis test was performed. Statistical significance was set as $p<0.05$.

RESULTS

Patient demographics

The mean age of the 25 patients was 34.80±16.75 years (mean±standard deviation), and that of the healthy controls was 35.6±11.0 years. Ages in the guttate group, the plaque group, and healthy controls were not significantly different (31.6±14.0 years vs. 38.0±9.2 years vs. 35.6±11.0 years, $p>0.05$). The mean duration of psoriasis for the 25 patients was 46.9±74.3 months. It was significantly longer in the plaque group (89.1±74.2 months) than in the guttate group (4.8±3.6 months, $p<0.05$; Table 2).

Comparison of cytokine mRNA profiles between patients with psoriasis and healthy controls

The blood mRNA levels of IL-2, IL-12p40, IFN-γ, IL-17A, IL-22, and IL-23R were analyzed in 25 patients with psoriasis and 5 healthy controls. As expected, the comparison between patients with psoriasis and healthy controls revealed that blood mRNA levels of IL-12p40, IL-17, and IL-22 were significantly elevated in psoriatic patients ($p<0.05$, Fig. 1). However, the levels of IL-2, IFN-γ, and IL-23R were lower in the psoriasis group ($p<0.05$, Fig. 1).

Comparison of cytokine mRNA profiles among healthy controls and patients with guttate or plaque psoriasis

In a comparison of the three groups, statistically significant differences in the mRNA levels of the six inflammatory cytokines were observed ($p<0.05$, Table 3). However the

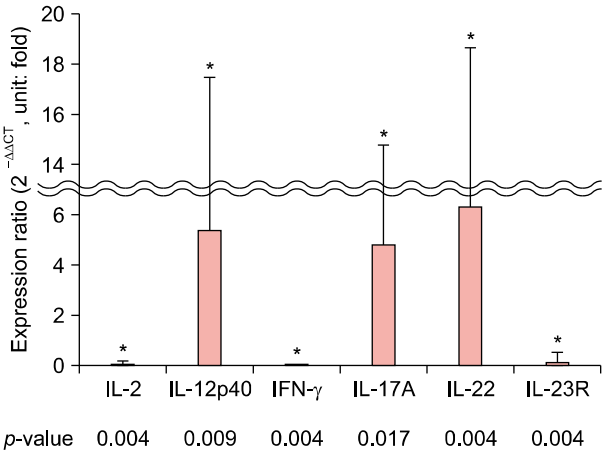

Fig. 1. Real-time reverse transcription polymerase chain reaction data on six inflammatory cytokine: The mRNA expression ratios ($2^{-\Delta\Delta CT}$) in patients with psoriasis compared to those in healthy controls were all significant (*$p<0.05$). The levels of interleukin (IL)-12p40, IL-17, and IL-22 were elevated in patients with psoriasis compared to those in healthy controls; however, the levels of IL-2, interferon (IFN)-γ, and IL-23R were lower in the psoriasis group.

Table 2. Demographics of healthy controls and patients with psoriasis

	Healthy controls	Patients with guttate psoriasis	Patients with plaque psoriasis	*p*-value
No. of case	5	10	15	
Age (yr)	35.6±11.0	31.6±14.0	38.0±9.2	0.204
Disease duration (mo)	N/A	4.8±3.6	89.1±74.2	0.003*

Values are presented as number only or mean±standard deviation. A Kruskal-Wallis test comparing ages of healthy controls and patients with guttate or plaque psoriasis, and a Mann-Whitney test comparing the duration of psoriasis between patients with guttate and plaque psoriasis were performed. N/A: not available. *$p<0.05$.

Table 3. Comparison of blood mRNA levels of cytokines between healthy controls and patients with guttate or plaque psoriasis

	Expression ratio ($2^{-\Delta\Delta CT}$, unit: fold)			*p*-value
	Control (n=5)	Guttate psoriasis (n=10)	Plaque psoriasis (n=15)	
IL-2	66.78±35.81	3.57±1.68	2.21±1.38	0.010*
IL-12p40	4.95±2.63	31.40±8.14	21.48±7.84	0.029*
IFN-γ	92.07±84.68	5.84±2.98	1.37±0.43	0.013*
IL-17A	0.78±0.51	2.40±1.88	2.17±2.01	0.036*
IL-22	4.69±3.53	18.35±8.60	13.25±6.39	0.022*
IL-23R	16.81±8.62	4.67±2.94	2.02±0.95	0.002*

Values are presented as mean±standard deviation. A Kruskal-Wallis test comparing the blood mRNA levels of inflammatory cytokines between healthy controls and patients with guttate or plaque psoriasis was performed. IL: interleukin, IFN: interferon. *$p<0.05$.

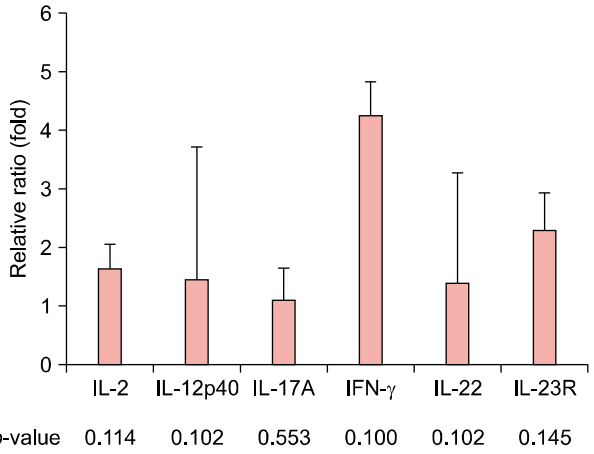

Fig. 2. Real-time reverse transcription polymerase chain reaction data on six inflammatory cytokine: The difference in the relative ratios of mRNA expression in patients with guttate and plaque psoriasis was not significant ($p>0.05$). IL: interleukin, IFN: interferon.

mRNA levels of inflammatory cytokines did not differ significantly between the guttate and plaque groups ($p>0.05$). Although the difference was not statistically significant in this study, blood IFN-γ and IL-23R levels in the guttate group were 4.3- and 2.3-fold higher than those in the plaque group (Fig. 2).

DISCUSSION

Psoriasis is a chronic inflammatory skin disease associated with the induction of Th1 and Th17 cell responses. Although there have been some studies with opposing results, the blood levels of Th1 and Th17 cytokines in patients with psoriasis are reported to be more increased than those of healthy controls[9,11,13-16]. The findings of the present study show that the gene expression levels of Th1-related and Th17-related cytokines are elevated in patients with psoriasis. We measured blood mRNA levels of six inflammatory cytokines, including Th1-related (IFN-γ, IL-2, and IL-12) and Th17-related (IL-17A, IL-22, and IL-23) cytokines, and analyzed them based on psoriasis phenotypes (guttate or plaque). As expected, the mRNA levels of IL-12p40, IL-17, and IL-22 were elevated in patients with psoriasis compared to those of healthy controls. However, intriguingly, the levels of IL-2, IFN-γ, and IL-23R were lower in the psoriasis group. Therefore, gene expression levels might be influenced by an unknown negative-feedback pathway or there might be a post-transcriptional defect in patients with psoriasis. In the case of IFN-γ, particularly, data on blood cytokine levels in psoriatic patients are controversial[6,17].

Psoriasis manifests in heterogeneous phenotypes, and its morphology may change through the course of the disease. Morphological differences in psoriasis cases are thought to be related to differences in the expression of inflammatory cytokines, particularly Th1-related and Th17-related cytokines. Previous evidence suggests that a change in inflammatory pathways, from IL-12/IFN-γ to IL-23/IL-17 or *vice versa*, can affect the psoriasis phenotype[5,9,14,18]. Christophers[5] proposed that activation of predominately Th1-related response results in a the stable plaque morphology, whereas Th17-related activation is associated with guttate or pustular psoriasis types. Our study, which compared the mRNA levels of blood inflammatory cytokines between patients with guttate and plaque psoriasis, revealed no statistically significant differences. A few studies compared blood cytokine levels between psoriasis subgroups; however, their results were contradictory and did not definitively determine whether there is a significant relationship between specific cytokines and phenotypes. Additionally, the results of our study show that the morphological phenotype may not be determined by activities of either the Th1 or Th17 pathway, specifically. Despite this, and even though the difference was not statistically significant, the blood mRNA levels of IFN-γ and IL-23R were higher in the guttate group than in the plaque group. Interestingly, and contrary to our expectations, mRNA levels of IFN-γ, a Th1-related cytokine, were lower in patients with plaque psoriasis than in those with guttate psoriasis. Thus, additional gene expression studies are needed to determine the association between Th1 or Th2 responses and psoriasis phenotype.

In summary, inflammatory cytokine mRNA levels in blood were significantly elevated in patients with psoriasis compared to healthy controls, although specific activities of either the Th1 or the Th17 pathway could not route the morphologic phenotype of psoriasis. A limitation of our study was the small number of patients involved; therefore, these results should be confirmed in future studies with larger sample sizes.

ACKNOWLEDGMENT

This work was supported, in part, by the Institute of Clinical Medicine Research of The Catholic University of Korea, Bucheon St. Mary's Hospital, Research Fund, 2016.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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